PHYLOGEOGRAPHY OF THREE WIDESPREAD NEOTROPICAL AVIAN TAXA: RUFOUS-TAILED HUMMINGBIRD, WHITE-BREASTED WOOD-WREN, AND *ANTHRACOTHORAX* MANGOS

By

Michael James Lelevier

RECOMMENDED:

Advisory Committee Chair

Richard D. Boone
Chair, Department of Biology and Wildlife

APPROVED:

Dean, College of Natural Science and Mathematics

Dean of the Graduate School

July 29, 2008
Date

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A

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Michael J. Lelevier, B.S.

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ABSTRACT

The three chapters presented in this thesis use molecular markers to examine the evolutionary history of three groups of widespread Neotropical birds. In chapter one, I found that *Amazilia tzacatl* forms a monophyletic clade and exhibits four genetic clades: Atlantic and Pacific slopes of Middle America, South America, and Isla Coiba. The Escudo Hummingbird (*A. t. handleyi*) is probably not a full biological species. Specimens from the eastern Darien province of Panama suggest that individuals from Middle and South America colonized this area within the past 25 years. In chapter two, I recovered an unresolved polytomy between *Henicorhina leucosticta* and its purported sister species, *H. leucoptera*. Mitochondrial DNA suggests a South American origin for *H. leucosticta-leucoptera* wood-wrens. In contrast to previous studies, I recovered high levels of structure among Middle American populations contradicting the hypothesis of a recent Middle American expansion. In chapter three, phylogenetic reconstructions support the merging of the genus *Eulampis* into *Anthracothorax*, but the inclusion of *Avocettula* is not supported. Biogeographically, ancestral area reconstructions support the radiation of *Anthracothorax* mangos out of the West Indies onto the mainland, which represents the first recognized example of mainland colonization by West Indian taxa for the family Trochilidae.

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PREFACE

The theme of this thesis was developed by my thesis advisor Kevin Winker (University of Alaska Department of Biology and Wildlife, University of Alaska Museum [UAM]), Mathew J. Miller, and myself. For all three chapters, I defined the questions, designed the studies, conducted all molecular and statistical analyses, and wrote the original drafts of the manuscripts.

For the first chapter, Co-authors John Klicka (Marjorie Barrick Museum), Eldridge Bermingham (Smithsonian Tropical Research Institute), and Patricia Escalante (Universidad Nacional Autónoma de México) provided indispensable specimens and comments to the final manuscript, as did co-authors Miller and Winker. For the second chapter, Andy Johnson (University of New Mexico), Rob Brumfield (Louisiana State University), Patricia Escalante, and Eldridge Bermingham provided specimens from Middle and South America. Co-authors Winker, Miller, and Bermingham made valuable contributions to the final manuscript. For the third chapter, Eldridge Bermingham and the Smithsonian Tropical Research Institute contributed the vast majority of specimens. Co-authors Miller and Winker made valuable contributions to the final manuscript.

A substantial portion of my lab work was conducted in the Bermingham lab of the Smithsonian Tropical Research Institute. While I collected most of the sequence data, Mathew Miller (UAM), Mersee-Madison Villar (UAM), Peggy Guitton Mayerma (STRI), and Maribel Gonzalez (STRI) all sequenced a considerable portion of the birds in this thesis. I am particularly grateful to Oris Sanjur (STRI) for her logistical, financial, and emotional support during my tenure at STRI. STRI's administrative staff provided

critical logistical support ranging from help with my immigration visa to collecting permits and use of the research vehicle and firearms.

Finally I would like to give my greatest thanks to Mathew Miller and Peggy Guitton Mayerma. During my time in Panama, Matt and Peggy were my sponsors, my lab managers, my neighbors, my emotional anchor, and my friends. My parents Michael and Kathy Lelevier have supported my academic dreams for almost a decade; thank you. Without their unwavering support I would never have been able to complete this thesis.

GENERAL INTRODUCTION

Phylogeography examines historic patterns of differentiation in relation to the geographic distribution of taxa (Avise 2000). This is accomplished by examining the current and historic distribution of a taxon in the context of its gene genealogy (Avise 2000). The integration of phylogenetics and geographic distribution enable us to infer past events such as population expansion, vicariance, migration, and population bottlenecks. In addition, phylogeographic methods can help managers prioritize areas of conservation importance by helping to define evolutionarily significant units (ESU; Moritz 1994).

Historic patterns of differentiation in the Neotropics are of particular interest due to the region's high levels of species diversity (Hillebrand 2004), its relative environmental stability (Bates et al. 1998), lower relative rates of evolution (Weir and Schluter 2004), and relatively recent major geological changes (e.g., Andean uplift: Gregory-Wodzicki 2000; closure of the Isthmus of Panama: Coates et al. 1992). Examination of a geographically broad sampling of a taxon's distribution using molecular methods offers the opportunity to study the patterns and possible mechanisms associated with this region's unique attributes.

The incorporations of samples throughout a taxon's known distribution also allows us to reassess phenotypically-based subspecific groups using phylogeographic methods, which is an integral part of properly identifying ESUs (Mortiz 1994; Zink 2004). For conservation purposes, the identification of subspecies based on phenotype alone might lead to the misallocation of conservation funding (Zink 2000; Zink 2004).

Thus, the use of both phenotypic and genotypic data in identifying taxa is important for the management and preservation of biological diversity.

My thesis uses phylogeographic and population genetic approaches to examine historic patterns of differentiation in three groups of widespread lowland Neotropical birds. The first and second chapters are single species phylogeographic studies, which use mitochondrial DNA (mtDNA) to examine both taxonomic and geographic structure throughout each taxon's distribution. The third chapter uses both nuclear and mtDNA to reconstruct the phylogeny of a widespread Neotropical hummingbird genus. In all three cases, I used reconstructed gene genealogies to identify expanding populations, geographically important geologic features, examine taxonomic relationships using molecular methods, and test phylogeographic hypothoses associated with each species. The results of these three studies should provide important baseline data and new insights into the phylogeography of lowland Neotropical birds.

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PHYLOGEOGRAPHY OF THE RUFOUS-TAILED HUMMINGBIRD (*AMAZILIA TZACATL*)[1]

The Rufous-tailed Hummingbird (*Amazilia tzacatl*) is a common resident of the Neotropical lowlands of Middle America and northern South America. It occurs from southeastern Mexico to southwestern Ecuador and western Venezuela, and five subspecies are recognized. Mitochondrial DNA (mtDNA) sequence variation from across most of the species' range showed four principle clades, three from Middle America and one from South America. The two main Middle American clades represent a relatively deep split (1.2%) between an Atlantic population that ranges from southeastern Mexico to central Panama and a population on the Pacific Slope of Panama. The third clade was made up mostly of birds from Isla Coiba, Panama; and the final clade, which differed from Middle American populations by 1.4%, was South American. The Escudo Hummingbird (*A. t. handleyi*), an endemic to Isla Escudo de Veraguas, Panama, and sometimes considered a separate species due to its larger size, did not have unique mtDNA haplotypes. Rather, *A. t. handleyi* was nested within the Atlantic Slope haplogroup. Specimens from the eastern Darien province of Panama suggest that this area was colonized within the past 25 years by individuals from the Pacific Slope (*A. t. tzacatl*) and South American populations (*A. t. jucunda*).

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[1] M. J. Lelevier, M. J. Miller, E. Bermingham, J. T. Klicka, P. Escalante, K. Winker. 2008. Prepared for The Condor. Phylogeography of the Rufous-tailed Hummingbird (*Amazilia tzacatl*).

INTRODUCTION

The Rufous-tailed Hummingbird (*Amazilia tzacatl*) is a common resident of the tropical and subtropical zones in the Neotropics, with a range that spans 28 degrees of latitude from southeastern Mexico to southwestern Ecuador and western Venezuela (Skutch 1931, Peters 1944, Schuchmann 1999). Within the hummingbird subfamily Trochilinae, *A. tzacatl* can be characterized as a generalist that inhabits a myriad of habitats, including humid forest edges, clearings, gardens, bushy coastal habitats, gallery and mangrove forests, and, rarely, dense forest or habitats above 1200 m (Skutch 1931, Wetmore 1968, Schuchmann 1999). Within its range, *A. tzacatl* is often the most commonly observed hummingbird (Skutch 1931, pers. obs.). Our study is the first to use molecular methods to examine the species' evolutionary history.

Currently, *A. tzacatl* is separated into five subspecies based on morphology and minor plumage variation. The nominate race, *A. t. tzacatl* (De la Llave 1832), is distributed from southern Mexico to the eastern Panama province of Panama (Weller and Schuchmann 1999, Schuchmann 1999). The range of *A. t. fuscicaudata* (Fraser 1840) extends from western Venezuela into northwestern Colombia (Schuchmann 1999). Traditionally, *A. t. fuscicaudata* has been merged into *A. t. tzacatl*, but Weller and Schuchmann's (1999) study of morphological variation demonstrated that the strictly South American *A. t. fuscicaudata* is significantly smaller than the Middle American race, *A. t. tzacatl*. *A. t. jucunda* (Heine 1863) is found from the Choco region of western Colombia to southern Ecuador (Schauensee 1948). The island endemic *A. t. handleyi* (Wetmore 1963), found only on Isla Escudo de Veraguas, Panama, was initially

described as an allospecies based on its considerably larger size and darker plumage (Wetmore 1959, Wetmore 1963), but recent literature has considered it a subspecies of *A. tzacatl* (Ridgely 1976, Schuchmann 1999). The fifth subspecies, *A. t. brehmi* (Weller and Schuchmann 1999), was recently described as endemic to the upper Rio Guiza valley in Nariño, Colombia. Finally, several surveys have demonstrated that a distributional gap exists between Middle and South American populations in the eastern Darien Province of Panama (Wetmore 1968, Robbins et al. 1985).

Within its Middle American populations, excluding the Isla Escudo form, *A. tzacatl* shows a clinal decrease in size with decreasing latitude, with the largest individuals found in Mexico (Weller and Schuchmann 1999). Within South American races, *A. t. fuscicaudata* also shows clinal variation in size, with southern populations slightly larger, and *A. t. jucunda* shows the opposite trend, with individuals from Colombia tending to be slightly larger than individuals from southern Ecuador (Weller and Schuchmann 1999). Individuals of the recently described *A. t. brehmi* differ significantly in size from adjacent populations of both *A. t. jucunda* and *A. t. fuscicaudata* (Weller and Schuchmann 1999). Although subspecies vary in size, only minor variations in plumage characters have been described; the species is rather uniform in appearance throughout its range (Wetmore 1963, Weller and Schuchmann 1999).

Based on plesiomorphic plumage characters and intrageneric relationships, Weller and Schuchmann (1999) postulated that the zoogeographic center of the species' range is located in northern Colombia. This was based on the observation that *A. t. fuscicaudata* represents the smallest subspecies of the group, sharing most of its plumage characters

with the remaining subspecies. In particular, the width and shape of the bronze-green margins of the rectrices remains the only described plumage character that distinguishes *A. t. fuscicaudata* from the other South American subspecies, and this character is shared with all Middle American taxa. Additional evidence given was that *A. tzacatl* is purported to form a superspecies with the range-restricted South American endemic *A. castaneiventris*, not as previously suggested with *A. rutila* and *A. yucatanensis*, which are restricted to Middle America (Schuchmann 1999). The predominance in South America of most of the species' subspecific variation also supports the notion of a South American origin. Weller and Schuchmann's (1999) hypothesis of a South American origin for the species is based solely on phenotypic and inferred intrageneric relationships; it has not been tested using molecular methods.

Here we investigate the magnitude and geographic structure of mitochondrial DNA (mtDNA) throughout the range of *A. tzacatl*. Genetic comparisons of Middle and South American populations will help identify historic patterns of differentiation, identify possible undescribed geographic variation, test Weller and Schuchmann's (1999) hypothesis of a South American origin for the species, and potentially identify contact zones between differentiated and expanding populations.

METHODS

TAXON SAMPLING

We sampled 95 individuals from 30 different localities within the range of *A. tzacatl*. We chose these localities to maximize geographic and subspecific coverage within the

species (Figure 1.1). We sampled one to ten individuals from each locality based on the availability of vouchered specimens. In addition to *A. tzacatl*, we obtained GenBank sequences of 20 closely related hummingbird species from seven genera for use as outgroups (McGuire et al. 2007). We obtained tissue samples from institutions listed in the Appendix (1.1).

AMPLIFICATION AND SEQUENCING

We extracted DNA from muscle tissue and feathers using the QIAamp DNA extraction kit (Qiagen) and, for feathers, a modified protocol from Taberlet and Bouvet (1991). We amplified the entire ND2 mitochondrial gene (1041 bp) via polymerase chain reaction using published and unpublished primers: L5215 (Hackett 1996), H6313 (Johnson and Sorenson 1998), and internal ND2-HUM525 (5'- CCGAAAAATCCTAGCCTTCT-3'). All amplifications used 20 µl reactions on an MJ Research Model PTC-200 Peltier thermal cycler and the protocols of Hackett (1996). We used electrophoresis on low-melting-point agarose gels to visualize PCR products. Typically, we observed a single amplification product, which was cut from the gel and cleaned using GELase™ Agarose Gel-Digesting Preparation and the "Fast Protocol" method (Epicentre Technologies, Madison, Wisconsin).

Purified amplification products were cycle-sequenced using the ABI Big Dye Terminator Cycle Sequencing Kit with QuiagenTaq Polymerase (Applied Biosystems, Inc., Forest City, CA) and the aforementioned primers for 25 cycles under the following conditions: 96° C for 10s, 50° C for 15s, and 60° C for 4 min. The cycle-sequencing

product was purified over Centri-Sep columns (Princeton Separations, Inc., Adelphia, NJ). Following ethanol precipitation, we resuspended sequencing products in Template Suppression Reagent (Applied Biosystems), and fragments were visualized on an ABI Prism 3130 automated sequencer (PE Applied Biosystems).

PHYLOGENETIC ANALYSES

We aligned sequence data by eye using Sequencher 4.6 (Gene Codes Corporation, Ann Arbor, MI). Only data with clean chromatograms with a high signal to noise ratio and without double peaks (which would indicate co-amplification of mitochondrial and nuclear loci) were used. Aligned sequences were visualized in MacClade 4.06 (Sinauer Associates, Inc., Sunderland, MA) to identify reading frames.

We reconstructed phylogenies using maximum likelihood (ML) methods in GARLI version 0.951 (Zwickl 2006) and Bayesian methods in MrBayes 3.1 (Ronquist and Huelsenbeck 2003). Following analyses using the data from 20 closely related hummingbird species, we designated *A. yucatanensis* and *A. rutila* as our outgroup taxa in all subsequent phylogenetic analyses.

We determined the model of molecular evolution for both ML and Bayesian analyses using the Akaike information criterion (AIC; Akaike 1973, Posada and Buckley 2004) in ModelTest (Posada and Crandall 1998). An HKY + G model of molecular evolution (Hasegawa et al. 1985) was selected as the best fit (-ln L = 3078.0569), with the following parameters: unequal base frequencies (A = 0.3175, C = 0.3323, and G =

0.1079), transition/transversion ratio = 12.6573, and shape parameter = 0.1069. We assessed node support in the likelihood tree using 1000 ML bootstrap iterations.

Bayesian analyses using the HKY + G model of molecular evolution had parameters estimated in a preliminary run using flat parameters. In addition, we also ran analyses under the HKY + specific sites (SS) model, in which the data were partitioned by codon site. We ran four Markov Chains for 10,000,000 generations, sampling one tree every 10,000 generations. Approximately 30,000 generations were required for the Markov chains to reach convergent and stable likelihood values. We therefore set the burnin to 30,000 and constructed a strict consensus tree using the remaining 997 trees in PAUP* (ver. 4b10, Swofford 1999). Node support in the Bayesian tree was assessed using posterior probabilities.

HISTORICAL DEMOGRAPHIC ANALYSES

We made inferences of demographic expansion using estimates of Fu's F_s and Romis-Onsins and Rozas' (2002) R_2, calculated using dnaSP 4.2 (Rozas et al. 2003). We applied these statistical analyses at several levels: Entire Range, Middle America, South America, island populations, and major clades resolved by phylogenetic analyses. Statistical significance values for the estimates were calculated based on 1000 coalescent simulations using a model of constant population size (Rozas et al. 2003). Mismatch distributions were also calculated to compare demographic histories of the major clades. We calculated the expected frequencies of the number of nucleotide substitutions under a model of rapid expansion and the frequencies of randomly chosen individuals differing

by a given number of nucleotides using dnaSP 4.0 (Rozas et al. 2003). Observed and expected frequencies were then plotted into mismatch distributions, and smoothness of the Poisson distributions were calculated using Harpending's (1994) raggedness index, which compares the observed and expected distributions to quantify the smoothness of the distribution of observed pairwise differences. We calculated statistical significance for these analyses based on coalescent simulations.

RESULTS

We obtained 1041 bp ND2 data from 95 individual *A. tzacatl* ranging from southern Mexico to south-central Ecuador and representing each of the three historically-accepted subspecies (Appendix 1.1). Fully 259 of 1041 (24.8%) sites were variable, and of these 131 (50.5%) were parsimony-informative; 52 unique haplotypes were present.

PHYLOGENETIC ANALYSES

Maximum likelihood analysis resulted in a single most likely tree (-ln L = -3301.4247), with *A. tzacatl* forming a monophyletic clade in relation to the outgroup taxa. As was previously established by McGuire et al. (2007), the genus *Amazilia* as currently recognized does not represent a monophyletic group. In the phylogeny produced by McGuire et al. (2007), *A. tzacatl* was placed sister to *A. rutila*, but their study did not include *A. yucatanensis*. When the latter was added to McGuire et al.'s (2007) ND2 data, ML analysis showed that the *A. tzacatl* clade was part of a strongly supported clade containing *A. yucatanensis* and *A. rutila*, with *A. tzacatl* and *A. yucatanensis* sister to one another (not shown). Within *A. tzacatl*, ML analysis resulted in three well-supported

(bootstrap $\geq$ 85%) clades (Pacific Slope of Panama, Atlantic Slope of Middle America, and South America) and a moderately supported Isla Coiba clade (64% bootstrap support; Figure 1.2). Relationships among these clades were not well resolved; they effectively comprised a polytomy.

The South American clade, encompassing the eastern Darien province of Panama and south-central Ecuador (Figure 1.2) was well supported, although its placement as sister to the rest of the group had low bootstrap support (51%). Due to lack of support this node was collapsed (Figure 1.2).

The well supported (85% bootstrap support) Pacific Slope clade was divided into two sister clades that differed by 0.67% uncorrected sequence divergence (Figure 1.2). One consisted of Pacific Panamanian populations (94% bootstrap support) from the Azuero Peninsula to the Burica Peninsula on the border of Costa Rica, and the other was represented by populations spanning the eastern Darien province of Panama.

The Atlantic Slope clade, representing populations from southern Mexico to central Panama, was well supported (94% bootstrap support). *A. t. handleyi,* endemic to Isla Escudo de Veraguas, Panama, formed a moderately supported (60%) monophyletic clade, but it was nested within the larger Atlantic Slope clade, differing by 3 fixed base pairs (bp; Figure 1.2). The Pacific Slope and Atlantic Slope clades differed by 1.2% uncorrected sequence divergence.

The remaining Isla Coiba clade was moderately supported (64% bootstrap support). In addition to including all the individuals from Isla Coiba, this clade also

included a single individual from the highlands of northern Costa Rica, but this clade did not include individuals from the adjacent mainland of Panama.

Bayesian analyses also recovered the four principle clades found in maximum likelihood (ML) analyses. Relationships among these clades were well supported, with posterior probabilities ranging from 0.90 to 1.0 (Figure 1.2). Topological differences between Bayesian and ML analyses only existed at more terminal, poorly-supported nodes. The differences occurring near branch tips were most likely due to few informative characters between closely-related haplotypes.

RANGE EXPANSION

Recent fieldwork by the University of Alaska Museum (UAM) and the Smithsonian Tropical Research Institute (STRI) has raised questions about the allopatric distribution of the Middle and South American populations of *A. tzacatl*. In 2003, two *A. tzacatl* were collected at Piñas Bay in the eastern Darien province of Panama. In 1945 and 1946, an extensive two-month survey of this locality did not find *A. tzacatl* (Wetmore 1946, 1959). In 2005, three *A. tzacatl* were collected at the Cana Ecotourism Lodge in the Darien. In 1982 a month-long survey of the avifauna in and around Cana did not find *A. tzacatl* (Robbins et al. 1985). Thus, the recently collected specimens appear to represent a range expansion into eastern Darien by *A. tzacatl*.

The five Darien specimens from the eastern edge of the province were split between the Pacific and South American clades and were 1.45% divergent (uncorrected sequence divergence). Thus, phylogenetic reconstructions showed that the Darien is

being colonized by both Middle and South American clades (Figure 1.2), with different individuals collected from a single locality representing the most divergent clades found in our study.

HISTORICAL DEMOGRAPHIC ANALYSES

Populations from mainland Middle America, representing specimens from both the Pacific and Atlantic slopes, comprised the only group that had significant ($P < 0.05$) negative Fu's F_s and R_2 values, suggesting a rapid demographic expansion. The non-significant values for the remaining groups suggested relatively stable demographic histories for these lineages (Table 1.1). In our analyses, the Pacific Slope group was the only one to have a significantly low ($P < 0.05$) raggedness value, suggesting a demographic expansion (Table 1.1). In addition, the mismatch distributions for the Middle American, Atlantic Slope, and Pacific slope groups were the only distributions showing the characteristics of a smooth Poisson distribution.

DISCUSSION

TAXONOMIC SIGNAL

Phylogenetic analyses recovered three well-supported clades and one moderately-supported clade. The relatively high level of divergence (1.4%) between Middle and South American clades is reflected in the recognized differentiation between the South American and Middle American subspecies *A. t. jucunda* and *A. t. tzacatl*.

The nominate subspecies (*A. t. tzacatl*), which is distributed throughout Middle America, did not form a monophyletic clade. Specimens representing *A. t. tzacatl* were included in three separate clades: Pacific Slope, Atlantic Slope, and Isla Coiba. *A. t. handleyi*, the Panamanian island endemic, represented a unique haplogroup, differing by 3 fixed bp differences from Atlantic Slope haplotypes of *A. t. tzacatl* (Figure 1.2). Because Isla Escudo de Veraguas only separated from the mainland of Panama 8,900 years ago (Summers et al. 1997), low levels of differentiation between *A. t. tzacatl* and *A. t. handleyi* were expected. Because mtDNA data do not constitute a test of subspecies validity (being unconnected to the phenotypic characters used to describe subspecies), we make no subspecific taxonomic recommendations from this study. We can observe, however, that present subspecific designations in Middle America do not adequately reflect the species' evolutionary history as revealed by mtDNA. Further population-level work on the Atlantic and Pacific slope populations with additional genotypic and phenotypic data could help clarify this apparent discrepancy.

PHYLOGENETIC STRUCTURE

Phylogenetic reconstruction placed *A. tzacatl* in a clade containing *A. yucatanensis* and *A. rutila*, both of which are restricted to Middle America (Schuchmann 1999). This association suggests a possible Middle American origin for *A. tzacatl*, but the inclusion of the purported sister species, *A. castaneiventris*, which was only recently rediscovered after 25 years (Cortes-Herrera et al. 2004), is needed to substantiate the sister relationship between *A. tzacatl* and *A. yucatanensis* suggested by our data.

Within *A. tzacatl*, phylogenetic reconstruction placed the South American subspecies *A. t. jucunda* sister to the entire group, although this placement was not well supported, and demographic analyses showed that specimens from mainland Middle America represented a demographic expansion. Sufficient specimens were not available to examine the genetic demographic history of the species' South American distribution; thus, we were unable to determine whether the South American populations were historically stable. The significant R_2 and Fu's F_s values from demographic analyses support Weller and Schuchmann's (1999) assertion that *A. tzacatl* originated in South America and subsequently expanded into Middle America; however intrageneric relationships may contradict the results of the demographic analyses. Thus, more extensive sampling within the species' South American distribution and the inclusion of *A. castaneiventris* will be required to fully test this hypothesis.

Specimens from Isla Coiba, off the Pacific coast of western Panama, comprised a unique clade that was least divergent (0.5%) from the Pacific Slope haplogroup and equally divergent (0.7%) from the South American and Atlantic Slope haplogroups. But the Isla Coiba clade included a single individual from the highlands of northern Costa Rica, with the remaining individuals from the Pacific slope of northern Costa Rica nested within the Atlantic Slope clade (Figure 1.2). The inclusion of this individual suggests the possible placement of this clade as sister to the Atlantic Slope. The Isla Coiba population may represent an early colonization of the Pacific slope by the species.

Evidence of morphological variation between the Pacific and Atlantic slopes of Middle America has yet to be described or observed, but the relatively high level of

genetic divergence (1.2%) suggests that the two populations, Atlantic and Pacific, have been isolated for a considerable period. Secondary contact between these two populations is seen only near Santa Fe, Veraguas, Panama, which lies within a low section of the Cordillera Central at 1200 m elevation. In addition, specimens from the lowlands of northern Costa Rica near the northern limits of the Cordillera Central were nested within the Atlantic Slope clade (Figure 1.2). The relatively high level of sequence divergence between specimens from the Atlantic and Pacific slopes, the occurrence of Atlantic Slope haplotypes at the northern extent of the mountain range, and the single area of secondary contact suggest that the Cordillera Central represents a fairly effective long-term barrier to gene flow in this species.

RECENT DARIEN RANGE EXPANSION

Our finding that recent specimens from the Darien represent both Middle and South American haplotypes suggests that an historic gap between these populations/subspecies has recently been colonized, bringing formerly allopatric populations into parapatry and even syntopy. This recent colonization of the densely forested eastern Darien by an open-country generalist may be correlated with forest cover change. Development of the Pan-American Highway has caused increases in logging and agriculture, and Dasmahapatra et al. (2002) suggested that this habitat change facilitated the movement of a hybrid zone between two *Anartia* butterfly species. Forests in Colombia have also undergone increased development; between 1930 and 1980, areas covered with pasture increased from 26% to 53% (Vina and Cavelier 1999). This suggests a trend toward conversion of

mature tropical forest into pastures for cattle and ranching. Changes in forest cover in both eastern Panama and Colombia may have facilitated *A. tzacatl's* recent colonization of the Darien.

In sum, *Amazilia tzacatl* is a monophyletic clade in relation to its closest relatives and exhibits four genetic clades: Atlantic and Pacific slopes of Middle America, South America, and Isla Coiba (the latter perhaps an early colonization of the Pacific Slope from the Atlantic Slope). To some extent the mtDNA clades corresponded with recognized subspecies, but there was disagreement as well, especially in the well-supported Atlantic and Pacific slope mtDNA clades, which are presently considered to be the nominate form *A. t. tzacatl*. The Escudo Island form is probably not a full biological species. Two contact zones are identified, both in Panama. One is between Atlantic Slope and Pacific Slope clades in Middle America, and the other, in the Darien, between Pacific Slope and South American clades; the latter appears to be recent. The syntopic occurrence in Darien, Panama of individuals from the deepest genetic split in the species suggests reproductive compatibility is retained between them. Thus, no cryptic species seem to occur within this taxon, neither phylogenetic nor biological.

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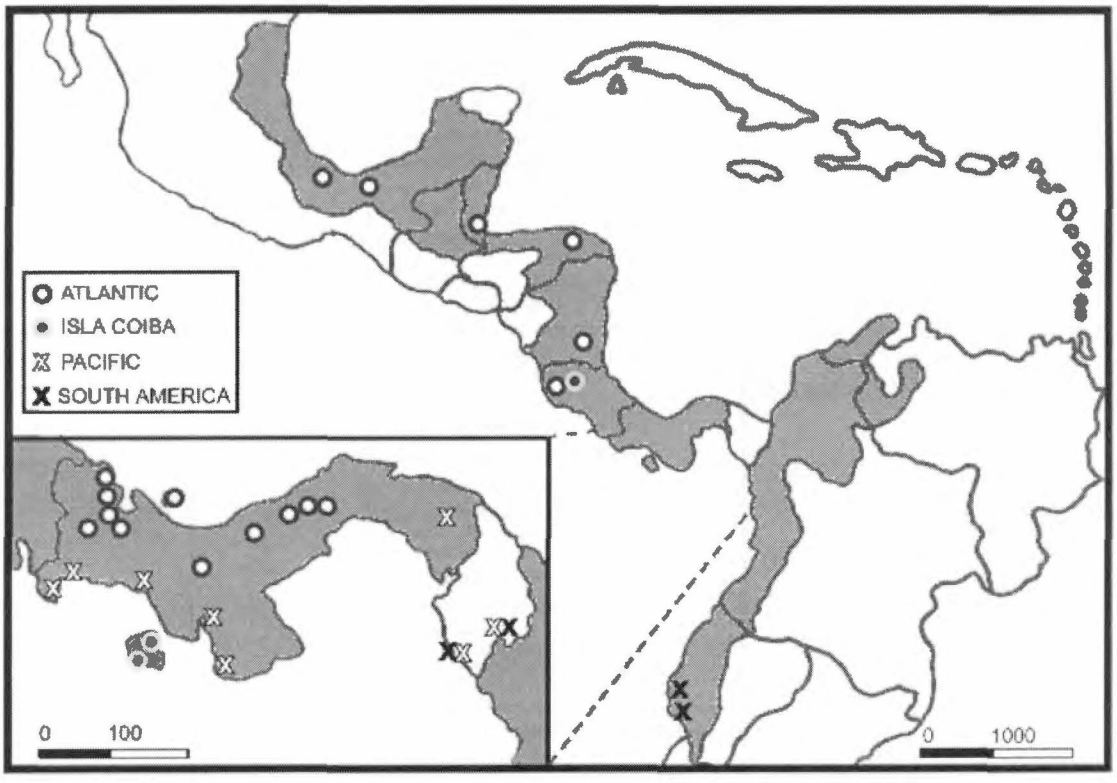

Figure 1.1 Geographic distribution of *Amazilia tzacatl.* Shaded areas delineate the species' range, and sampling localities are represented by the symbols in the legend, with each symbol corresponding to clades recovered in phylogenetic reconstructions.

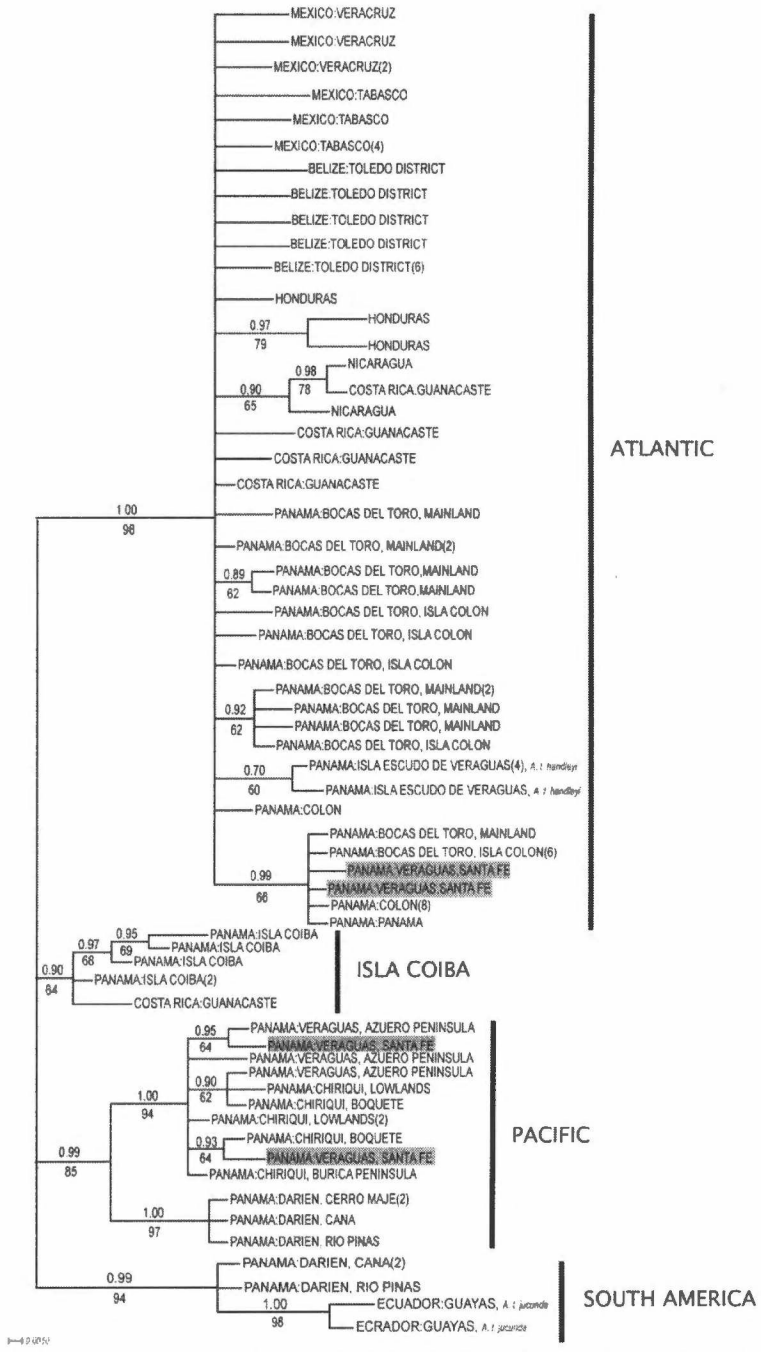

Figure 1.2 Bayesian phylogeny for *Amazilia tzacatl.* Bayesian consensus tree of *Amazilia tzacatl* reconstructed using 1041 bp of the mtDNA ND2 gene. Numbers above the branches indicate all posterior probabilities ≥ 0.70, and numbers below the branches indicate bootstrap values from ML analyses. All individuals are the nominate subspecies (*Amazilia t. tzacatl*), unless otherwise indicated. Shaded individuals represent individuals obtained from a contact zone between Pacific and Atlantic Slope clades.

Table 1.1 Results of historical demographic analyses for *Amazilia tzacatl.* Results of historical demographic analyses calculated using dnaSP v.4.2 (Rozas et al. 2003). Groups used in analyses represent geographic areas of interest. Raggedness values (r) are measurements of the smoothness of the mismatch distributions. R_2 and Fu's F_s are measurements of population stability. Significance was determined based on 1000 coalescent simulations under a model of constant population size.

Phylogeographic Group	n	Raggedness (r)	P (r)	R_2	P (R_2)	Fu's F_s	P (Fu's F_s)
Middle America	90	0.0177	0.139	0.04	< 0.04	-3.19	< 0.02
Atlantic Slope of Middle America	58	0.0469	0.276	0.05	< 0.01	-15.68	< 0.01
Pacific Slope of Middle America	14	0.0499	0.001	0.14	< 0.05	-1.61	< 0.05
South America	5	0.2300	0.453	0.25	> 0.50	1.22	> 0.50
Isla Coiba	5	0.1300	0.133	0.25	> 0.40	-1.01	> 0.10
Isla Escudo de Veraguas	5	0.2000	0.087	0.40	> 0.70	-0.77	> 0.10

Appendix 1.1. Taxon, museum of origin, specimen voucher number, and geographic origin for birds in this study. Museum abbreviations are as follows: ANSP, Academy of Natural Sciences of Philadelphia; FMNH, Field Museum of Natural History; LSU, Louisiana State University Museum of Natural Science; UNLV, University of Nevada, Las Vegas, Marjorie Barrick Museum of Natural History; UAM, University of Alaska Museum; UWBM, University of Washington Burke Museum.

Taxon	Museum	Voucher Number	Geographic Origin
A. t. tzacatl	UAM	UAM10510, CAM350, CAM376, CAM324, UAM10508, CAM399	Mexico:Tabasco
A. t. tzacatl	UAM	TUX1120, PEP2504, PEP2505, PEP2512	Mexico:Veracruz
A. t. tzacatl	UAM	UAM8037, UAM14312, UAM14322, UAM14313, UAM14461, UAM14513, UAM7963, UAM9079, UAM9203, UAM9237	Belize:Toledo District
A. t. tzacatl	UNLV	MBM9003, MBM9004, MBM9076	Honduras
A. t. tzacatl	UNLV	MBM4520, MBM4521	Nicaragua
A. t. tzacatl	HMCZ	336161, 336162, 335741, 335804, 335575	Costa Rica:Guanacasta
A. t. tzacatl	UNLV	JK06-222, JK06-138, GMS1994, JK06-143, JK06-217, JMD758, JMD766, MBM18342	Panama:Bocas del Toro
A. t. tzacatl	STRI	JTW231	Panama:Bocas del Toro
A. t. tzacatl	UAM	UAM24460, UAM24401, UAM24396, UAM24400, UAM24397, UAM24408, UAM24399, UAM24461, UAM24247, UAM24640	Panama:Bocas, Isla Colon
A. t. handleyi	STRI	MJL030, AWK3260, AWK3265, AWK3269 AWK3278	Panama:Isla Escudo de Veraguas
A. t. tzacatl	UAM	UAM20618, UAM20629, UAM20372, UAM19208, UAM24462, UAM22692, UAM20299, UAM24403, UAM24591, UAM22693	Panama:Panama
A. t. tzacatl	UNLV	JMD896	Panama:Chiriqui, Burica
A. t. tzacatl	UAM	UAM24409, UAM24404	Panama:Chiriqui, Boquete
A. t. tzacatl	UNLV	GMS2201	Panama:Chiriqui lowlands
A. t. tzacatl	UAM	UAM24410, UAM24458	Panama:Chiriqui lowlands
A. t. tzacatl	UNLV	MBM15840, MBM15673, MBM15841	Panama:Azucro Peninsula
A. t. tzacatl	UNLV	MBM15866, MBM15867, MBM16129, MBM14971	Panama:Santa Fe
A. t. tzacatl	LSU	B46732, B46653, B46696, B46680, B46664	Panama:Isla Coiba
A. t. tzacatl	UNLV	GMS1887, GMS1947	Panama:Darien
A. tzacatl	LSU	JTW610, JTW721	Panama:Darien, Pinas Bay
A. tzacatl	UAM	UAM22691, UAM24255, UAM22690	Panama:Darien, Cana
A. t. jucunda	ANSP	ANSP 3333, ANSP3638	Ecuador
OUTGROUP			
A. amabilis	LSUMZ	B-28483	Panama:Colon
A. decora	LSUMZ	B-100024	Costa Rica:Puntarenas
A. chionogaster	LSUMZ	B-17165	Argentina
A. edward	UAM	UAM22684	Panama:Chiriqui
A. fimbriata	LSUMZ	B-5956	Ecuador:Morona-Santiago
A. franciae	LSUMZ	B-12063	Ecuador:Pichincha
A. rutila	UWBM	UWBM56002	Nicaragua:Puerto Cabezas
A. saucerrotei	FMNH	FMNH393025	Costa Rica:Guanacaste
A. versicolor	FMNH	FMNH395409	Brazil:Sao Paulo
A. viridigaster	LSUMZ	B-7490	Venezuela:Amazonas
A. yucatanensis	UAM	TUX1316	Mexico:Veracruz
Aphantochroa cirrochloris	FMNH	FMNH399167	Brazil:Pernambuco
Chrysuronia oenone	LSUMZ	B-5318	Peru:San Martin
Damophila julie	LSUMZ	B-16556	Panama:Panama
Hylocharis cyanus	LSUMZ	B-9547	Bolivia:Pando
H. eliciae	LSUMZ	B-16074	Costa Rica:Puntarenas
H. grayi	ANSP	ANSP5064	Equador:Imbabura
H. sapphirina	LSUMZ	B-12912	Bolivia:Santa Cruz
Lepidopyga coeruleogularis	LSUMZ	B-29053	Panama:Cocle
Taphrospilus hypostictus	LSUMZ	B-5465	Peru:San Martin

THE WHITE-BREASTED WOOD-WREN (*HENICORHINA LEUCOSTICTA*) SHOWS HIGH LEVELS OF PHYLOGEOGRAPHIC STRUCTURE THROUGHOUT THE NEOTROPICS[1]

The White-breasted Wood-Wren (*Henicorhina leucosticta*) is a common lowland resident of Middle and northern South America, with a range extending from southern Mexico to southwestern Ecuador and from French Guiana and northern Brazil to Peru. Using the mitochondrial ND2 gene, we reconstructed phylogenetic relationships of the *Henicorhina* wood-wren complex. Mitochondrial DNA (mtDNA) sequence variation from across the species' range showed three principal clades, one Middle American and two South American (Choco and Amazon-Darien). These three clades were well supported and highly divergent (9.1-11.4%) in mtDNA. The Choco clade, representing samples from the Choco region of northwestern Ecuador, was sister to the Bar-winged Wood-wren (*H. leucoptera*). The Amazon-Darien clade was relatively deeply split (4.4%) from Amazonian and eastern Panamanian specimens, with specimens occurring east of the Andes showing relatively shallow genetic splits (1-2%). In all phylogenetic reconstructions, the Middle American clade showed high levels of geographic structure, with three well-supported subclades (Panama, Belize-Honduras, and Mexico). In general, Middle American *H. leucosticta* populations are characterized by large phylogenetic splits (2.5-4.9%) between geographically proximate populations. The largest

[1] M. J. Lelevier, M. J. Miller, E. Bermingham, R. Brumfield, P. Escalante, K. Winker. 2008. Prepared for The AUK. The White-breasted Wood-wren (*Henicorhina leucosticta*) shows high levels of phylogeographic structure throughout the Neotropics.

phylogenetic break in Middle America (9.1%) occurred between central and eastern Panamanian populations.

INTRODUCTION

The White-breasted Wood-Wren (*Henicorhina leucosticta*) is a common lowland resident of Middle and northern South America, with a range extending from southern Mexico to southwestern Ecuador and from French Guiana and northern Brazil to northeastern Peru (Figure 2.1: Skutch 1960; del Hoyo et al. 2005; Restall et al. 2006). Throughout its range, *H. leucosticta* is a forest-dwelling insectivore typically restricted to wet lowland forest from sea level to 1800 m, but it is usually found below 1000 m (Skutch 1960; del Hoyo et al. 2005; Restall et al. 2006). Although common, *H. leucosticta* wood-wrens are often difficult to observe because they spend their lives on or near the ground in dense forests, rarely ascending more than 2 m above the ground (Skutch 1960; per obs.).

Thirteen subspecies of *H. leucosticta* are currently recognized (Dickinson 2003; del Hoyo et. al. 2005), several of which are distinguished by subtle plumage differences (Dickerman 1973; del Hoyo et al. 2005; Restall et al. 2006). The subspecies of *H. leucosticta* have been grouped, based on geography, into two general groups: a Middle American-Choco group spanning the length of Middle America into western Ecuador, and an Amazonian group that includes populations in northeastern Peru, Ecuador, Colombia, and Venezuela, through most of the Guyana Shield into northern Brazil. It has been suggested that these groups, between which the Andes likely form a barrier, may warrant species status (Winker et al. 1996, Dingle et al. 2006).

Recent molecular work within the family Troglodytidae has helped elucidate relationships within *Henicorhina* (Dingle et al. 2006; Mann et al. 2006). Historically, the Bar-winged Wood-wren (*H. leucoptera*) has been placed as sister to the Gray-breasted Wood-wren (*H. leucophrys*), with both species considered to be sister to *H. leucosticta* (Fitzpatrick et al. 1977). The inferred sister relationship between *H. leucoptera* and *H. leucophrys* was based on behavioral and morphological similarities between the taxa, although *H. leucoptera* is phenotypically intermediate between *H. leucophrys* and *H. leucosticta* in some characters (Fitzpatrick et al. 1977). Based on mtDNA, a paraphyletic relationship was recovered between *H. leucosticta* and *H. leucoptera* by Dingle et al. (2006), contradicting the previously accepted relationship. In addition, a well supported sister relationship between *H. leucosticta* and *H. leucophrys* has been demonstrated by multiple studies (Dingle et al. 2006; Mann et al. 2006). Therefore, it seems that *H. leucosticta* may not be a monophyletic species, but the paraphyletic relationship recovered by Dingle et al. (2006) included three unvouchered *H. leucoptera* samples, so identification cannot be verified.

The intraspecific relationships of *H. leucosticta* found by Dingle et al. (2006) largely adhered to previous, phenotypically-based subspecific groupings, although their study split South American samples into two distinct groups occurring on the eastern and western sides of the Andes. Their phylogenetic reconstructions, based on mtDNA, recovered three distinct clades (Middle American, Amazon, and Choco) with the Amazon and Middle American clades sister to a Choco-*H. leucoptera* clade (Dingle et al. 2006). This reconstruction split the Middle American-Choco subspecific grouping into two

relatively deeply divergent clades. Large levels of genetic divergence between clades led Dingle et al. (2006) to suggest splitting *H. leucosticta* into three taxonomic species. Furthermore, Dingle et al. (2006) suggested that the negligible differentiation between Belizean and Panamanian populations indicated that Middle American populations have either undergone a recent range expansion or are subject to long-distance gene flow. Inferences made by Dingle et al. (2006) were limited both by their geographic sampling and by the use of non-vouchered material. Their study only included eighteen samples from three countries, representing only five of the 13 recognized subspecies. The lack of both taxonomic and geographic coverage for the species increased the likelihood of their missing important genetic diversity, such as intermediate haplotypes between divergent clades.

Here we investigate the geographic structure of mitochondrial DNA (mtDNA) variation among Middle and South American populations of *H. leucosticta.* The incorporation of a more intensive sampling scheme, both geographically and taxonomically, than that of Dingle et al. (2006) enabled us to examine whether previous interspecific relationships are robust, identify historic patterns of differentiation, possibly identify undescribed geographic variation, test Dingle et al.'s (2006) hypothesis of a recent Middle American expansion, and use molecular dating methods to put differentiation among clades into a chronological framework.

METHODS

TAXONOMIC SAMPLING

We sampled 87 individuals from 18 localities within the range of *H. leucosticta.* We chose localities that maximized both our geographic and taxonomic (subspecific) coverage. Our sample distribution included 10 of the 13 currently recognized subspecies from eight countries. For each locality, we sampled 1-12 individuals based on availability of vouchered specimens. In addition to *H. leucosticta*, we included samples of *H. leucoptera*, *H. leucophrys*, and *Thryothorus ludovicianus* for use as outgroups (Appendix 2.1).

AMPLIFICATION AND SEQUENCING

Total genomic DNA was extracted from tissue samples using Qiagen DNeasy tissue kits following the manufacturer's protocol (Qiagen, Valencia, California). We amplified the second subunit of the NADH dehydrogenase gene (1041 bp) via polymerase chain reaction (PCR) using published primers: L5215 (Hackett 1996), H6313 (Johnson and Sorenson 1998), and MetB (Miller et al. 2007). All amplifications were performed using 20 μL reactions and previously published protocols (Hunt et al. 2001).

We visualized PCR products using electrophoresis on low-melting-point agarose gels. Amplification products were excised from the gel and cleaned using GELase™ Agarose Gel-Digesting Preparation and the "Fast Protocol" method (Epicentre Technologies, Madison, Wisconsin). Sequencing of purified PCR products was performed using a Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Inc.,

Forest City, CA) with 2 μL of Big Dye and 20-40 ng of purified PCR product. Sequencing reactions were purified over Centri-Sep columns (Princeton Separations, Inc., Adelphia, NJ) and analyzed with an ABI Prism 3130 automated sequencer (Applied Biosystems, Inc.).

Overlapping sequences were aligned by eye using Sequencher 4.6 (Gene Codes Corporation, Ann Arbor, MI). Only clean chromatograms without double peaks and a high signal-to-noise ratio were used. Final alignments were visualized in MacClade 4.06 (Sinauer Associates, Inc., Sunderland, MA) to identify reading frames.

PHYLOGENETIC ANALYSES

We reconstructed phylogenies using maximum likelihood (ML) methods in GARLI version 0.951 (Zwickl 2006) and Bayesian methods in MrBayes 3.1 (Ronquist and Huelsenbeck 2003). In all phylogenetic analyses we designated *Thryothorus ludovicianus* as our outgroup (Mann et al. 2006).

We determined the most appropriate model of molecular evolution for both ML and Bayesian analyses using the Akaike information criterion (AIC; Akaike 1973, Posada and Buckley 2004) as implemented in MrModeltest v2.2 (Nylander 2004). The results of the AIC indicated that the GTR + G model best fit the data. We assessed node support in the likelihood tree using 1000 ML bootstrap iterations.

Using GTR + G, we ran four Markov Chains for 10,500,000 generations, sampling one tree every 10,000 generations. Approximately 50,000 generations were required for the Markov Chains to reach convergent and stable likelihood values. Taking

a cautious approach, we set our burnin to 500,000 and constructed a 50% majority-rule consensus tree using the remaining 1000 trees. Node support in the Bayesian consensus tree was assessed using posterior probabilities.

HISTORICAL DEMOGRAPHIC ANALYSES

We made inferences of demographic expansion using estimates of Fu's F_s and Romis-Onsins and Rozas' (2002) R_2, calculated using dnaSP 4.4 (Rozas et al. 2003). We applied these analyses at multiple levels: entire range, Middle America, western Ecuador, Amazon, and individual subclades recovered by phylogenetic analyses. Statistical significance for the estimates was calculated using 1000 coalescent simulations under a model of constant population size (Rozas et al. 2003).

MOLECULAR DATING

To evaluate the timing of major divergence events within *Henicorhina*, we analyzed our data using molecular clock methods. To test the assumption of a molecular clock, we examined our data using a likelihood ratio test as implemented in PAUP* (ver. 4b10, Swofford 1999).

The use of a molecular clock in dating divergence events has many possible sources of error: clock calibration, saturation, and rate variation to name a few (Edwards and Beerli 2000, Arbogast et al. 2002, Ho et al. 2007). In a single-locus study, the error associated with estimating divergence dates may be particularly troublesome. Given these

problems, time estimates for divergence events should be considered relative approximations.

Dates were estimated using a rate calibration of cytochrome *b* (cyt *b*) from Hawaiian honeycreepers (Fleischer et al. 1998). The applicability of this calibration in the family Troglodytidae was shown by Barker (2007). The original rate calibration was 1.6% under a Kimura model of molecular evolution (Fleischer et al. 1998), and the rate was recalibrated to 2.2% under the more complex GTR + I model (Weir and Schluter 2004). For all divergence date estimates we used the recalibrated rate of 2.2%. Because this calibration was based on cyt *b* and our dataset is exclusively ND2, we used a range of rate estimates as our confidence interval. This interval included Fleischer et al.'s (1998) calibration (1.6%) and Dacosta and Klicka's (2008) recalibration of the cyt *b* clock for ND2 in the genus *Trogon* (3.41%), which was similar to previous relative rate calibrations in a variety of birds (Klicka et al. 2000, Ribas et al. 2005, Sheldon et al. 2005).

RESULTS

Excluding our outgroup taxa, 798 (76.6%) characters were constant, 84 (8%) were variable but uninformative, and 159 (15.2%) were parsimony informative. Fully 47 individuals (54%) of the 87 total specimens had unique haplotypes, with 33 (70%) haplotypes occurring in Middle America ($N = 61$) and 14 (30%) in South America ($N = 26$).

PHYLOGENETIC RELATIONSHIPS

Our phylogenetic analyses recovered highly congruent topologies. Topological inconsistencies between ML and Bayesian analyses only existed among terminal nodes within clades representing a single geographic area (not shown). All phylogenetic reconstructions identified a sister relationship between *H. leucophrys* and a paraphyletic clade containing all individuals of *H. leucosticta* and *H. leucoptera* (Figure 2.2). Haplotypes between these two groups were separated by high levels of sequences divergence (13.0 – 14.6% uncorrected). The majority of deep internodes linking highly differentiated populations or species were well supported by high posterior possibilities (0.91-1.0) and bootstrap values (most 100%). The only two exceptions occurred in the clade containing individuals of *H. leucosticta* from western Ecuador and individuals of *H. leucoptera* (posterior probability 0.76 and bootstrap value of 75%) and in the clade containing the only individual from the Pacific slope of Costa Rica (posterior probability of 0.63 and bootstrap value of 59%). Due to low support values, these nodes were collapsed (Figure 2.2).

Within the *leucosticta-leucoptera* clade, the sister relationship between birds from western Ecuador and individuals of *leucoptera* was poorly supported (Figure 2.2), rendering *leucosticta* paraphyletic with respect to *leucoptera.* Although paraphyletic, high levels of sequence divergence exist between the two taxa (9.0-10.6% uncorrected), with moderate levels of sequence divergence also occurring between the two subclades of *leucoptera* (4.2-4.4% uncorrected).

Mitochondrial DNA haplotypes within *H. leucosticta* as presently recognized grouped into three clades: 1) a Choco clade including individuals from northwestern Ecuador; 2) a Middle American clade from southern Mexico to Central Panama; and 3) an Amazon-Darien clade comprised of populations from northeastern Peru, eastern Ecuador, Guyana, and eastern Panama (Figure 2.2).

The *leucosticta* birds from the Choco region of Ecuador were 9.7-11.5% divergent from Middle American birds and 9.1-10.2% divergent from Amazon-Darien birds. The Middle American and Amazon-Darien clades were more closely allied, differing by 7.1-9.2%, and geographically-proximate populations from the Canal Zone of central Panama and the eastern Darien province of Panama showed relatively high levels of divergence (9.0-9.2% uncorrected; Figure 2.2). Individuals from the Canal Zone occurred mostly with the rest of the Middle American birds, whereas Darien individuals occurred with the Amazonian birds. A single Canal Zone (central Panama) individual fell out with the Amazonian haplotype clade (Figure 2.2).

High levels of structure were seen among Middle American populations, with individuals sorting into four well-supported haplogroups (Veracruz, Tabasco, Belize-Honduras, and Panama) and one poorly-supported haplogroup (Costa Rica; Figure 2.2). Haplogroups within the Middle America clade were separated by moderate levels of sequence divergence (1-5%).

HISTORIC DEMOGRAPHIC ANALYSES

Statistical estimates of Fu's F_s and R_2 at all levels returned non-significant values ($P > 0.05$). Non-significant estimates suggest relatively stable demographic histories for all lineages (Table 2.1).

MOLECULAR DATING

The likelihood ratio test failed to reject the assumption of a molecular clock (-2ln L = 8.76, d.f. = 96, $p = 0.53$). Based on a molecular clock of 2.2%, *H. leucophrys* diverged from the *leucoptera-leucosticta* clade approximately 7.32 Mya (4.58-9.52 Myr). *H. leucoptera* diverged from the rest of the *leucosticta* clade approximately 6.16 Mya (4.48-9.52 Myr). The remainder of the *leucosticta* clades diverged 2.31-5.39 Mya (1.68-8.33 Myr; Table 2.2).

DISCUSSION

PHYLOGENETIC AND BIOGEOGRAPHIC RELATIONSHIPS

The well-supported sister relationship between *H. leucophrys* and the *leucosticta-leucoptera* clade was recovered by all reconstructions. The divergence event between these two clades likely occurred prior to the final uplift of the Andes (~2.7 Mya; Gregory-Wodzicki 2000) and the closure of the Isthmus of Panama (~3 Mya; Coates et al. 1992); both of these events occurred well after the estimated date of divergence between these clades and the confidence interval for this date estimate.

An unresolved polytomy between *H. leucosticta* and *H. leucoptera* was recovered by all analyses, with overlapping divergence date estimates between *leucoptera* (6.16 Mya), western Ecuador (5.75 Mya), and the remaining *H. leucosticta* clades. The data suggest that populations of *H. leucoptera* diverged before the western Ecuadorian clade, but overlapping confidence intervals made it unlikely that the sequence of divergence events can be determined from these data. Divergence estimates for *H. leucoptera* and the Ecuadorean *H. leucosticta* clade fell before the completion of the Andean uplift, suggesting that the formation of the Andes may have contributed to the relatively deep level of divergence between these two clades and between these and the remaining *H. leucosticta* clades. In the case of *Henicorhina leucosticta-leucoptera* wood-wrens, all Middle American individuals were nested among South American haplogroups (Dingle et al. 2006; this study). Both the nested position of Middle American birds and the estimated divergence dates suggest a South American origin for this group.

Demographic analyses indicated a relatively stable history for the western Ecuadorian clade. While the two populations of *H. leucoptera* showed moderate levels of divergence between haplogroups, the estimated divergence date between these populations suggested that it occurred after the Andean uplift. Divergence dates suggested that segregation on high-elevation mountaintop islands may have caused the differentiation between these *leucoptera* clades. In the case of western Ecuadorian *H. leucosticta*, the sedentary nature of the species (del Hoyo et al. 2005) along with segregation by the Andean chain likely contributed to the deep divergences recovered between western Ecuador and populations occurring east of the Andes. Our lack of

specimens from adjacent Colombian populations and the lack of phenotypic studies examining the species throughout its range limit our ability to determine relationships between major phylogroups where they likely come into contact in northwestern South America.

Excluding those from western Ecuador, all *H. leucosticta* in our study occurred in a single clade (Figure 2.2, Table 2.2) which was subdivided into two sister clades (Amazon-Darien and Middle America). Divergences between these subclades likely occurred prior to the formation of the Isthmus of Panama and Andean uplift (Figure 2.2, Table 2.2). The subsequent colonization of Middle America likely occurred after the completion of these major geological events (Figure 2.2, Table 2.2). Our divergence estimate of ~2.48 Mya put the split between the Amazonian-Darien and Middle American groups after the final uplift of the Andes. These estimates suggest that Amazonian populations crossed over or went around the Andes after completion of the range's uplift to colonize Colombia and southern Middle America, which was also recently documented in a *Mionectes* flycatcher (Miller et al. 2008). Darien populations (which are geographically Middle American) represent a deeply divergent clade (9.0-9.2%) when compared to populations from the rest of Middle America (Figure 2.2). A single individual with a Darien haplotype was found in the Canal Zone of central Panama. This bird was phenotypically similar to the Central Panama group but genotypically a member of the Darien group, and it may be a hybrid between the two groups.

In contrast to Dingle et al. (2006), we recovered five clades within Middle America, four of which were well supported. Demographic analyses of Middle America and its associated subclades suggested a relatively stable demographic history for the region. This contradicts Dingle et al.'s (2006) assertion that Middle American populations represented a recent expansion or an example of long-distance gene flow. The relatively deep levels of divergence among clades (1-5%), together with support for a stable demographic history and the sedentary nature of the species (del Hoyo et al. 2005) suggest a long history for the species in Middle America. Colonizing populations likely inhabited this region and remained sedentary. The structure apparent in our data between such populations as Veracruz, Tabasco, and Belize is surprising given that there are no obvious major vicariance barriers separating them. An in-depth examination of Middle American populations is needed to assess the factors contributing to the geographic partitioning of genetic variation among them. The same is true of the multiple contact zones inferred by these data.

TAXONOMIC RECOMMENDATIONS

It is quite possible that more than one biological species is involved in the *H. leucosticta* group. However, without specimens from Colombia, where individuals of the Ecuador and Amazon-Darien clades meet, it is not possible at present to infer species limits. As our specimens from the Canal Zone in Panama show, gene flow seems to be occurring between rather distinct phylogenetic clades. Given the polytomous relationship between *leucosticta* and *leucoptera* and the inability to diagnose species limits based on mtDNA

alone, this presents a quandary. Although monophyly could be achieved by relegating *leucoptera* to being a subspecies of *leucosticta* (Figure 2.2), we suggest instead that further work be done to determine species limits among the four main clades of the *leucoptera-leucosticta* complex.

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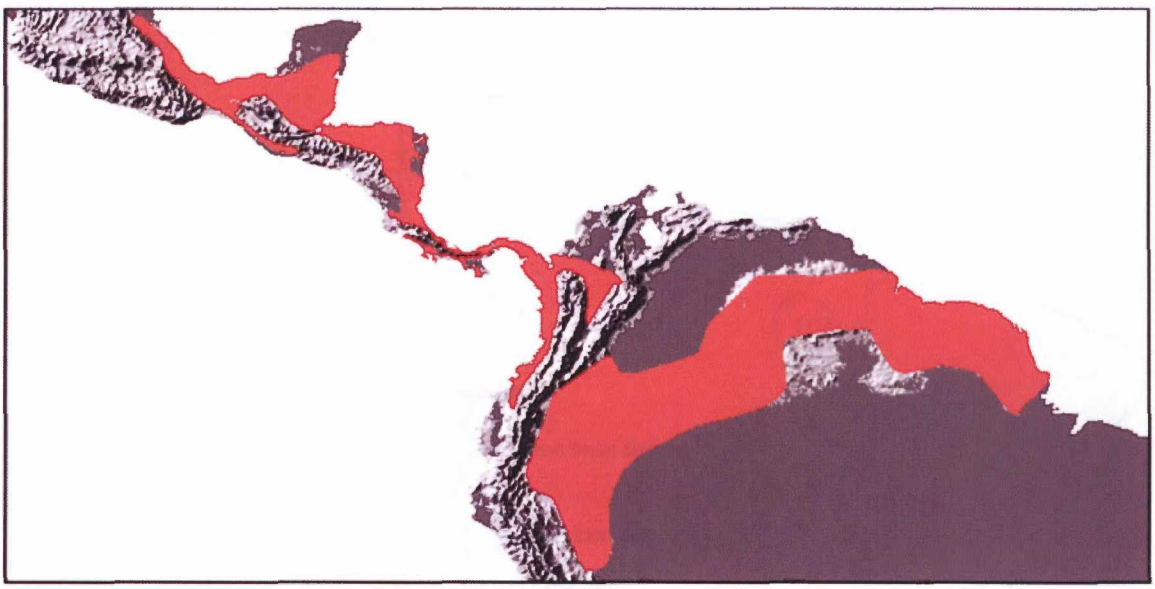

Figure 2.1 Distribution of the White-breasted Wood-wren (*Henicorhina leucosticta*).

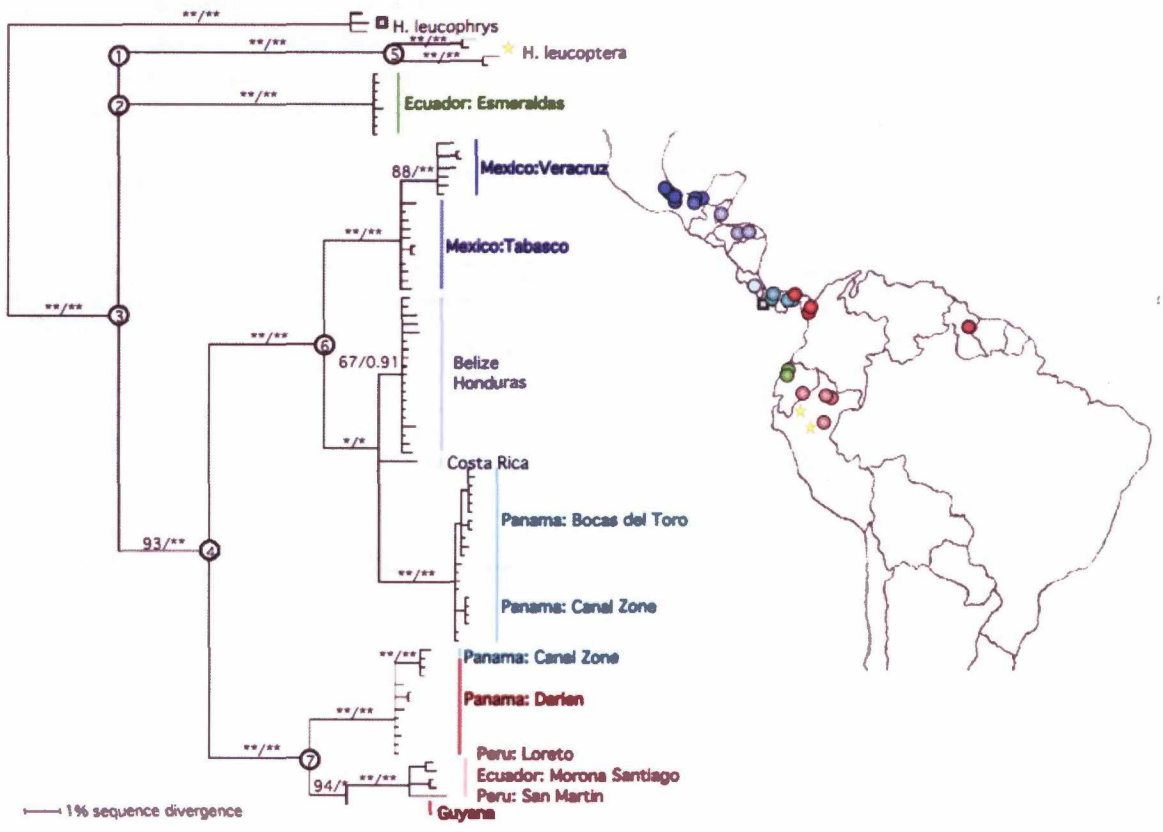

Figure 2.2 Bayesian phylogeny for *Henicorhina leucosticta.* Majority-rule consensus Bayesian topology for 94 individuals of *Henicorhina* wood-wrens (87 *H. leucosticta*, 4 *H. leucoptera*, and 3 *H. leucophrys*) based on 1041 bp of the complete mitochondrial ND2 gene. Root (not shown) is to *Thryothorus ludovicianus*. First number above the branch indicates the Maximum Likelihood bootstrap values, and the second number indicates Bayesian posterior probability. Double asterisks indicate support values of 100% (or 1.00), and single asterisks denote support values of 95% (or 0.95) or greater. Numbered nodes refer to nodes dated in subsequent analyses (Table 2.2). The map shows the collection localities for all individuals, color coded to correspond to major clades from the tree. Symbols on the map correspond to the species in the tree: Circles are *H. leucosticta*, stars are *H. leucoptera*, and the square is *H. leucophrys*.

Table 2.1 Results of historical demographic analyses for *Henicorhina leucosticta*. Results of historical demographic analyses calculated using dnaSP v. 4.2 (Rozas et al. 2003). Groups analyzed represent major clades from phylogenetic analyses (Figure 2.2). S denotes the number of variable sites, and *H* indicates haplotype diversity for the associated group. R_2 and Fu's F_s are measurements of population stability. Significance of demographic statistics was estimated using 1000 coalescent simulations under a model of constant population size.

Phylogeographic Group	N	S	h	Hd	*SD* (Hd)	Fu's F_s	*P* (Fu's F_s)	R_2	*P* (R_2)
Middle America	61	196	33	0.96	0.01	0.36	*P* > 0.7	0.083	*P* > 0.2
Tabasco	11	10	8	0.95	0.05	-4.01	*P* > 0.8	0.091	*P* > 0.1
Veracruz	7	3	3	0.67	0.16	0.11	*P* > 0.8	0.246	*P* > 0.6
Belize-Honduras	19	16	11	0.79	0.10	-6.49	*P* > 0.8	0.067	*P* > 0.1
Panama	22	181	11	0.91	0.03	4.65	*P* > 0.7	0.120	*P* > 0.4
Amazon-Darien	18	79	11	0.93	0.04	3.41	*P* > 0.7	0.149	*P* > 0.1
Darien	12	8	5	0.83	0.07	0.81	*P* > 0.8	0.183	*P* > 0.1
Amazon	6	49	6	1.00	0.10	-0.19	*P* > 0.6	0.226	*P* > 0.7
Western Ecuador	8	2	3	0.46	0.20	-1.00	*P* > 0.9	0.217	*P* > 0.3

Table 2.2 Estimated ages of divergence events in the *Henicorhina* phylogeny (Figure 2.2).

Node	Age[1]	CI[2]
1	6.16	(4.48-9.52)
2	5.75	(4.18-8.89)
3	7.32	(5.32-11.31)
4	5.39	(3.92-8.33)
5	2.48	(1.80-3.83)
6	2.31	(1.68-3.57)
7	2.42	(1.76-3.74)

[1] Estimated ages in millions of years based on a 2.2% per million years molecular clock.

[2] CI estimated ages based on 1.6% and 3.4% per million years molecular clock.

Appendix 2.1. Taxon, museum of origin, specimen voucher number, and geographic origin for birds in this study. Museum abbreviations are as follows: CNAV, Colección National de Aves, Instituto de Biología, Universidad Nacional Autónoma de México; LSUMZ, Louisiana State University Museum of Natural Science; UNLV, University of Nevada, Las Vegas, Marjorie Barrick Museum of Natural History; STRI, Smithsonian Tropical Research Institute; UAM, University of Alaska Museum; and UWBM, University of Washington Burke Museum

Taxon	Museum	Museum Voucher Number	Geographic Origin
Thryothorus ludovicianus	UWBM	EV1294	Mexico
Henicorhina leucophrys	UAM	UAM24664, UAM24663	Panama:Chiriqui
H. leucophrys	LSUMZ	B28281	Panama:Chiriqui
H. leucoptera	LSUMZ	B43581, B43608	Peru:Loreto
H. leucoptera	LSUMZ	B39918, B39925	Peru:San Martin
H. leucosticta prostheleuca	CNAV	GLS278, MGL78, TUX230, PEP2506,	Mexico:Veracruz
H. leucosticta prostheleuca	UAM	UAM20910, UAM21094, UAM7146	Mexico:Veracruz
H. l. decolorata	CNAV	CAM447, CAM360, CAM375, CAM389, CAM415, CAM417	Mexico:Tabasco
H. l. decolorata	UAM	UAM11996, UAM11999, UAM11998, UAM12000, UAM11997	Mexico:Tabasco
H. l. smithei	UAM	UAM24662, UAM9233, UAM9232, UAM9069, UAM22731, UAM24323, UAM14319, UAM14318, UAM22731, UAM24659	Belize:Toledo
H. l. tropaea	UNLV	GAV1744, GAV1743, GMS197, GAV1457, GAV1742, JK99_081, GAV1745, GMS169, GMS112	Honduras
H. l. costaricensis	LSUMZ	B35756	Costa Rica:Cartago
H. l. pittieri	UNLV	GMS2007, JK06_125, JK06_130, JMD754, GMS2006, JK06_124	Panama:Bocas del Toro
H. l. pittieri	STRI	JTW280, JTW203, JTW319, JTW089,	Panama:Bocas del Toro
H. l. pittieri	UAM	UAM20578, UAM20625, UAM22765, UAM24661, UAM22727, UAM22728, UAM22726, UAM24580, B28784, UAM24660	Panama:Canal Zone
H. l. pittieri	STRI	STRIX193	Panama:Canal Zone
H. l. darienensis	UAM	JTW728, UAM24476, UAM22768, UAM22770, UAM22762, UAM22767, UAM22766, UAM22761, UAM22769	Panama:Darien
H. l. darienensis	LSUMZ	B1357, B2097, B2236	Panama:Darien
H. l. inornata	STRI	MJM023	Ecuador:Esmeraldas
H. l. inornata	LSUMZ	B11739, B11756, B11867, B12005, B11738, B11868, B11869	Ecuador:Esmeraldas
H. l. hauxwelli	LSUMZ	B42866, B43460, B5391	Peru:Loreto
H. l. hauxwelli	LSUMZ	B5992, B6019	Ecuador: Morona Santiago
H. l. leucosticta	LSUMZ	B48436	Guyana:Karaudanawa

OUT OF THE WEST INDIES: BIOGEOGRAPHY AND SYSTEMATICS OF THE WIDESPREAD HUMMINGBIRD GENUS *ANTHRACOTHORAX*[1]

There are seven species of *Anthracothorax* hummingbirds, and they range from southern Mexico to the lowlands of South America and most of the West Indies. We sampled mitochondrial DNA (mtDNA) and nuclear DNA sequences from a geographically broad sampling of the taxa in this genus. Results suggest at least three changes to the group's taxonomy: The endemic West Indian genus *Eulampis* is deeply nested within *Anthracothorax*; the monotypic genus *Avocettula*, recently considered a member of *Anthracothorax*, was instead sister to *Chrysolampis*; and the enigmatic Veraguan Mango (*Anthracothorax veraguensis*) formed a monophletic group with *A. prevostii*. Additionally, our phylogeny indicated that diversification within *Anthracothorax* may have originated in the West Indies, with subsequent colonization of mainland Central and South America. The Black-throated Mango (*A. nigricollis*) and Green-breasted Mango (*A. prevostii*), which have the most extensive distributions in the genus, showed extremely low levels of sequence divergence (< 0.002%) between geographically distant populations.

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[1] M. J. Lelevier, M. J. Miller, E. Bermingham, K. Winker. 2008. Prepared for Molecular Phylogenetics and Evolution. Out of the West Indies: biogeography and systematics of the widespread hummingbird genus *Anthracothorax*.

INTRODUCTION

The family Trochilidae, hummingbirds, is an ideal group in which to examine the biogeographical history of the directionality of mainland-to-island (or island-to-mainland) colonization. Hummingbirds, with some ~331 recognized species in ~104 genera (Schuchmann 1999, Dickinson 2003), represent the second largest avian family in the New World. This strictly New World family has undergone a dramatic adaptive radiation, with species distributed from Alaska to the southern tip of South America, including an extensive West Indian distribution (Schuchmann 1999; Dickinson 2003). Currently, 18 extant species of hummingbirds (representing 10 genera) are distributed throughout the West Indies. Of these, 15 species (in six genera) are endemic to these islands.

The colonization of mainland areas by island fauna is generally considered rare (Brown and Lomolino 1998; Cox and Moore 2005), although some examples have been described (Hedges 1989; Hedges et al. 1992; Seutin et al. 1994; Seidel 1996; Raxworthy et al. 2002; Townsend and Larson 2002; Nicholson et al. 2005). The converse, the colonization of islands from the mainland, has been demonstrated repeatedly, with classic examples among the flora and fauna of the Galapagos, Hawaii, and Krakatau (Brown and Lomolino 1998; Cox and Moore 2005 and references therein).

Traditionally, the colonization of the West Indies by mainland hummingbird fauna is considered to have been one-way (mainland-to-island), with the West Indies failing to contribute its own endemically-derived taxa to other regional faunas (Bleiweiss 1998a). This view, originally based on a 28-species DNA-hybridization study (Bleiweiss

1998a,b), has been subsequently supported by several studies incorporating more extensive taxon sampling (75-151 species) and the use of both mitochondrial (mt) and nuclear DNA (Altshuler et al. 2004; McGuire et al. 2007). Hummingbird research, current and historic, has thus far provided no examples supporting mainland colonization by West Indian taxa, although such contributions have been described in other groups (passerines: Seutin et al. 1994; lizards: Nicholson et al. 2005; frogs: Hedges 1989; Hedges et al. 1992; and turtles: Seidel 1996). To test the hypothesis of a one-way colonization of the West Indies by hummingbirds and to determine relationships among taxa in the group, we reconstructed the phylogeny of the widespread hummingbird genus *Anthracothorax* (Figure 3.1).

STUDY TAXA

Anthracothorax, the mangos, are common lowland residents of the Neotropical and subtropical zones, with distributions that collectively range from southern Mexico to Paraguay and southern Brazil and most of the Greater Antilles (Schuchmann 1999; Dickinson 2003). *Anthracothorax* currently includes seven species, three of which are endemic to the West Indies. In addition, the distributions of *A. nigricollis* and *A. prevostii*, the two mainland taxa with the most extensive ranges, extend into the islands of the West Indies (Schuchmann 1999; Dickinson 2003). This widespread distribution suggests that *Anthracothorax* mangos are particularly adept at colonizing a wide variety of habitats and islands, and this ability may have contributed to the success of *Anthracothorax* within the West Indies (Bleiweiss 1998a).

The systematics of this hummingbird genus are poorly understood (Olson 1993). In addition to testing biogeographic hypotheses, a full phylogenetic examination of *Anthracothorax* would help resolve several confusing inter- and intra-generic relationships associated with the genus. Among these ambiguous relationships is the proper generic placement of the monotypic genus *Avocettula recurvirostris*. The reclassification of *Avocettula recurvirostris* as *Anthracothorax recurvirostris* was proposed by Schuchmann (1999) and followed by Dickinson (2003) on the grounds that aside from its uniquely recurved bill, *Avocettula* is similar to *Anthracothorax* in both plumage and nest structure. Plumage similarities were further supported by Schmitz-Ornes's (2006) study, which showed complete overlap in ultraviolet color spectral data between the two genera. In 2004, the South American Checklist Committee (SACC) re-split the two genera based on a lack of studies examining their genetic and morphological relationships (Stiles 2004).

Among the species of *Anthracothorax* with widespread mainland distributions, Schuchmann (1999) asserted that *A. prevostii*, *A. nigricollis*, *A. veraguensis*, and more than likely *A. viridigula*, form a superspecies, yet the specific species status of *A. veraguensis* is an uncertain matter. *A. veraguensis*, a restricted-range Panamanian endemic, was originally described as a subspecies of *A. prevostii* (Wetmore 1968). This was based on three specimens that showed small amounts of black on the throat, which is a distinguishing characteristic of *A. prevostii*. Olson (1993) suggested that this distinction is uncertain. According to Olson (1993), two of Wetmore's specimens do not show marked differences from specimens of *A. veraguensis*, and the third specimen was later

identified as an *A. nigricollis* hybrid. Based on these findings, The North American Checklist Committee elevated *A. veraguensis* to species level in 1995, yet until now this distinction is based solely on plumage characters and the allopatric distribution of the two taxa (AOU 1995). Phylogenetic examination of Panamanian populations of *A. nigricollis*, *A. prevostii*, and *A. veraguensis* could help resolve this question.

Here we use both mitochondrial and nuclear DNA to reconstruct phylogenetic relationships among all of the traditionally accepted species within the genus *Anthracothorax*, including representation from other genera purported to be closely allied to the group. A resolved phylogeny will allow us to test the biogeographic hypothesis of whether the genus arose in the West Indies or on the mainland, determine the proper generic placement of the genus *Avocettula* and the placement of *A. veraguensis*, and clarify other relationships in this poorly understood genus.

METHODS

TAXON SAMPLING

Ingroup sampling for our study included all seven representatives of the genus *Anthracothorax*, including subspecific coverage of all but three narrow-range *A. prevostii* subspecies (Appendix 3.1; Schuchmann 1999; Dickinson 2003). We also sampled three closely-related genera (*Avocettula*, *Chrysolampis*, and *Eulampis*), based on purported relationships from previous phenotypic and molecular studies, along with our outgroup *Andron aequatorialis* (McGuire et al. 2007). For each taxon, we sampled 1-5 individuals per locality from up to seven different localities. We chose these localities to maximize

subspecific and geographic coverage within each species. Many of the West Indian samples were unvouchered (Appendix 3.1).

AMPLIFICATION AND SEQUENCING

Genomic DNA was extracted from muscle tissue using Qiagen DNeasy tissue kits following the manufacturer's protocol (Qiagen, Valencia, CA). We amplified the entire second subunit of the mitochondrial nicotinamide adenine dinucleotide dehydrogenase gene (ND2, 1041 bp) for all 62 samples (Appendix 3.1). Based on the absence of large phylogenetic breaks within each taxon, we chose a subsample of 1-3 individuals from each of our ingroup and outgroup species for further sequencing. For our truncated dataset, we included two additional mitochondrial genes: subunits 6 and 8 of the adenosine triphosphate gene (ATP-6 and -8, 842 bp), the first subunit of the cytochrome oxidase gene (COI-barcode, 617 bp), and two nuclear introns: the fifth intron of the nuclear ß-fibrinogen gene (BFib5, 582 bp) and the fifth intron of the nuclear adenylate kinase gene (AK1, 541 bp). We used the following primers for both amplification and sequencing: ND2-L5215 (Hacket 1996) and H6313 (Johnson and Sorenson 1998); ATP-6 and -8-COIIGQL, COIIIHMH (Joseph et al. 2004); COI-barcode- BirdF1, BirdR1 (Herbert et al. 2004); BFib5- FIB5L, FIB5H (Driskell and Christidis 2004); and AK1-AK5b+, and AK6c- (Shapiro and Dumbacher 2001). All amplifications used 20 µL reactions on an MJ Research Model PTC-200 Peltier thermal cycler using previously described protocols (Hunt et al. 2001).

We visualized polymerase chain reaction (PCR) products using electrophoresis on low-melting-point agarose gels. Amplification products were cut from the gel and cleaned using GELase™ Agarose Gel-Digesting Preparation and the "Fast Protocol" method (Epicentre Technologies, Madison, WI). Sequencing of purified PCR products was performed using an ABI Big Dye Terminator Cycle Sequencing Kit with QuiagenTaq Polymerase (Applied Biosystems, Inc., Forest City, CA) and the aforementioned primers. Cycle-sequencing products were run on an ABI Prism 3130 automated sequencer (PE Applied Biosystems).

PHYLOGENETIC ANALYSES

We used the program Sequencher 4.6 (Gene Codes Corporation, Ann Arbor, MI) to align sequences. We only used data with clean chromatograms with a high signal-to-noise ratio. The ATP-6 and -8 genes had an overlap of 10 bp. To maintain the first position reading frame in both genes, we duplicated the overlap using protocols from Hunt et al. (2001). The aligned intron sequences (BFib5 and AK1) contained several inferred insertions or deletions (indels), but alignment of these sequences was straightforward and done by eye. Heterozygous sites were scored using ambiguity codes, and we excluded base positions contributing to length polymorphisms. We visualized the final aligned sequences in MacClade 4.06 (Sinauer Associates, Inc., Sunderland, MA) to identify reading frames.

Phylogenetic reconstructions were performed using Bayesian methods in MrBayes 3.1 (Huelsenbeck and Ronquist 2001). For all analyses *Andron aequatorialis* was designated as our outgroup taxon.

We determined the best model of molecular evolution for Bayesian analyses using the Akaike information criterion (AIC; Akaike 1973, Posada and Buckley 2004) as implemented in the program MrModeltest v2.2 (Nylander 2004). The following models were selected as best fits: GTR + I + G (ND2), GTR + G (ATP-6 and -8), GTR + I + G (COI-barcode), HKY + I + G (BFib5), and HKY + I (AK1).

Although our five datasets (ND2, ATP-6 and -8, COI-barcode, Bfib5, and AK1) were initially analyzed individually (including both the full and subsampled ND2 datasets), our goal was to analyze the entire subsampled mitochondrial dataset and the entire subsampled mitochondrial and nuclear dataset jointly. We used Bayes factors, calculated using the harmonic mean from the sump command in MrBayes, to choose among partitioning schemes (Bradley et al. 2005).

Bayes factor analysis provided strong evidence that a five-partition model (representing each gene) was more appropriate than a one-partition (all data together), two-partition (mtDNA vs nuclear), or three-partition (mtDNA and both nuclear introns) model (one-partition: -lnL= 6405.24; two-partition: -lnL= 5526.63; three-partition: -lnL= 5525.92; five-partition: -lnL= 5332.26).

For each analysis, we ran four Markov chains for 15,000,000 generations, sampling one tree every 10,000 generations. For the full ND2 analysis, approximately 50,000 generations were required for the Markov chains to reach convergent and stable

likelihood values, whereas the partitioned analyses required approximately 1,000,000 generations to reach stable likelihood values. For each of our analyses, we took a cautious approach and removed more samples than needed (100,000 for ND2 and 1,500,000 for partitioned analyses) to ensure that burnin runs were not included in our sampled trees. The remaining 1490 trees from the full ND2 dataset and 1350 trees from the partitioned analyses were used to create 50% majority rule consensus trees. Node support values in the Bayesian trees were assessed using posterior probabilities.

ANCESTRAL AREA ANALYSIS

Using the 50% majority rule phylogram from the combined ND2, ATP-6 and -8, and COI-barcode dataset, we reconstructed the ancestral area of the genus *Anthracothorax* using maximum parsimony and maximum likelihood ancestral state simulations in Mesquite v. 2.01 (Maddison and Maddison 2005) using the default maximum likelihood model for character reconstructions. To examine the basal nodes in more detail, we used a Bayesian tree-sampling methodology (Lutzoni et al. 2001; Pagel and Lutzoni 2002), in which character histories were simulated over the final 1000 post- burnin trees from our Bayesian analysis by the program Mesquite (Maddison and Maddison 2005). For all reconstructions, terminal taxa were coded as either mainland or West Indian based on sample locality.

We examined alternative topologies consistent with a mainland origin for the genus using MrBayes 3.1 (Huelsenbeck and Ronquist 2001). We designated our alternative topologies (1. monophyly of West Indian clade; 2. monophyly of mainland

and West Indian clades) using MacClade 4.06 (Sinauer Associates, Inc., Sunderland, MA), and we used the filter constraint command to identify trees from our 1000 post-burnin trees from our Bayesian analysis that were consistent with the alternative topologies.

RESULTS

PHYLOGENETIC ANALYSES

Monophyly of the genus *Anthracothorax* was not supported by any of our analyses. In all reconstructions, both species of the West Indian endemic genus *Eulampis* were nested within *Anthracothorax* (Figures 3.2, 3.3). This topology contradicts the previously well-supported sister relationship between the two genera (Altschuler et al. 2004; McGuire et al. 2007). The sister relationship between *Avocettula recurvirostris*, *Chrysolampis mosquitus*, and the paraphyletic clade containing all species of the genera *Eulampis* and *Anthracothorax* was strongly supported in all reconstructions (Figures 3.2, 3.3).

Within the *Anthracothorax* + *Eulampis* clade, the sister relationship between *A. dominicus* and the remaining members of *Eulampis* and *Anthracothorax* was well supported. With the remaining species falling into two clades, the first well-supported clade consisted of West Indian endemics (*A. viridis*, *Eulampis jugularis*, and *E. holosericeus*), and the remaining clade included all of the mainland species (*A. nigricollis*, *A. viridigula*, *A. prevostii*, and *A. veraguensis*) and the West Indian endemic *A. mango*. Although the placement of *A. mango* as sister to all of the mainland species

was well supported in both mtDNA analyses, it was poorly supported (posterior probability of 0.64) in the combined nuclear and mtDNA dataset (Figures 3.2, 3.3).

The monotypic genera *Avocettula* and *Chrysolampis*, represented here by the species *Chrysolampis mosquitus* and *Avocettula recurvirostris*, received significant support in all analyses as sister taxa to all extant species of the genus *Anthracothorax*, with the latter being sister to the entire group (Figures 3.2, 3.3).

Our full ND2 dataset included 62 individuals from 12 taxa (11 ingroup and one outgroup). Monophyly of each ingroup taxon was strongly supported (posterior probabilities of 1.0) in all taxa except *A. veraguensis*, which formed a monophyletic clade with *A. prevostii* (Figure 3.2). Although these closely-related taxa did not share a common haplotype, the putative species *A. veraguensis* only differed from *A. prevostii* by two fixed base pairs.

West Indian taxa showed the highest levels of sequence divergence in ND2 (1.15% uncorrected), which occurred between the two recognized subspecies of *A. dominicus* on the islands of Hispaniola and Puerto Rico (410 km apart). Mainland taxa with the largest geographic distributions (*A. prevostii* and *A. nigricollis*) showed low levels of sequence divergence between geographically distant localities. Samples of *A. nigricollis* from Panama and Guyana (~2000 km apart) shared a common haplotype, while individuals from Panama and Bolivia (~2800 km apart) showed only two base-pair differences (Figure 3.2). Samples of *A. prevostii* representing three distinct subspecies from Belize, Honduras, and Panama showed only a single base-pair difference between the three haplotypes (Figure 3.2).

ANCESTRAL AREA ANALYSIS

Both maximum parsimony and maximum likelihood ancestral state reconstructions supported a West Indian ancestor for mainland members of the genus *Anthracothorax* (when considering the genus sensu lato; i.e., including *Eulampis*). Reconstructions using the Bayesian tree-sampling method recovered all basal nodes in at least 95% of the post-burnin trees (Figure 3.4; A: 100%, B: 98%, C: 96%, and D: 95%). The majority of trees reconstructed a mainland origin for nodes A and B (A: 100%, B: 56%; Figure 3.4), whereas nodes C and D were reconstructed as having a West Indian origin in nearly three quarters of the trees (C: 74%, D: 73%; Figure 3.4).

Searches of the post-burnin trees using alternative topologies returned zero trees indicating monophyly of both West Indian and mainland clades. Three trees out of 1000 returned topologies consistent with a monophyletic West Indian clade nested within a larger mainland clade, indicating an extremely small probability for this relationship.

DISCUSSION

TAXONOMIC RELATIONSHIPS

Taxonomic relationships within the family Trochilidae, which includes ~65 monotypic genera, have been complicated by the use of phenotypic characters such as plumage to establish taxonomic status. The inter- and intra-generic relationships of the genus *Anthracothorax* appear to be a prime example of the perils associated with using plumage and ecology, in the absence of molecular data, to infer generic and species-level relationships in this family.

The endemic West Indian genus *Eulampis* currently includes two species, *E. jugularis* and *E. holosericeus*. Based on phenotypic differences, these two species were previously classified as monotypic genera, *Eulampis jugularis* and *Sericotes holosericeus* (Schuchmann 1999, Dickinson 2003). Recent taxonomic treatments, based on phenotypic and molecular studies, have supported both the merging of *Sericotes* into *Eulampis* and a sister relationship between *Eulampis* and *Anthracothorax* (Bleiweiss 1998a,b; Schuchmann 1999; Dickinson 2003; Altshuler et al. 2004; McGuire et al. 2007).

In all of our phylogenetic reconstructions, the genus *Eulampis* was deeply nested within *Anthracothorax*. This relationship contradicts the seemingly well-supported sister relationship shown by recent molecular studies (Bleiweiss 1998a,b; Altshuler et al. 2004; McGuire et al. 2007). The topological differences between our study and previous ones are likely due to our more complete taxon sampling. Previous studies have only sampled one or two species of *Anthracothorax*, and in each case the species sampled belonged to a single clade (Bleiweiss 1998a,b; Altshuler et al. 2004; McGuire et al. 2007).

Aside from described plumage differences (Schuchmann 1999; Schmitz-Ornés 2006), the two genera (*Eulampis* and *Anthracothorax*) share several morphological and ecological similarities. Morphologically, both genera are relatively large (6-10 g) with similarly decurved bills with minute serrations on the tomia (Ornelas 1994; Schuchmann 1999). Ecologically, both genera are edge and open-country specialists known to hawk insects and rob nectar (Ornelas 1994; Schuchmann 1999).

The monotypic genus *Avocettula* has previously been merged into the genus *Anthracothorax* on the basis of plumage and nest structure similarities (Schuchmann

1999; Dickinson 2003; Schmitz-Ornés 2006). Our data do not support the merging of *Avocettula* and *Anthracothorax*. In all of our phylogenetic reconstructions *Avocettula* was sister to the monotypic genus *Chrysolampis* rather than to *Anthracothorax*. Even though there is significant overlap in plumage characteristics between *Anthracothorax* and *Avocettula* (Schmitz-Ornés 2006), the two genera differ morphologically. Bill structure is the most striking difference between them. The recurved tip of *Avocettula recurvirostris*'s bill is unique to the species; no hummingbird shares a similar characteristic (Schuchmann 1999). In addition, *A. recurvirostris* is on average only 4 g, about 2 g smaller than the smallest members of the genus *Anthracothorax*, which makes it similar in size to its apparent sister species *Chrysolampis mosquitus.*

In the cases of both *Eulampis* and *Avocettula*, the use of a few phenotypic characters to delineate generic limits led to erroneous relationships. In the case of *Eulampis*, phenotypic dissimilarity caused members of the genus to be placed first into two separate monotypic genera, and then to be merged into a single genus inappropriately separated from its closest relatives. Our study clearly places *Eulampis* within the genus *Anthracothorax*. To re-establish monophyly of the genus *Anthracothorax*, we recommend that *Eulampis* be merged into *Anthracothorax*. In the case of *Avocettula*, phenotypic similarity inappropriately led to its merging with *Anthracothorax*. Our study instead places *Avocettula* as sister to *Chrysolampis.* This relationship is also supported by morphological characters, suggesting that the classification of *Avocettula recurvirostris* as *Anthracothorax recurvirostris* is incorrect.

Within the genus *Anthracothorax*, the species status of the Panamanian endemic Veraguan Mango (*A. veraguensis*) has been contentious. Prior to 1995, *A. veraguensis* was classified as a subspecies of *A. prevostii* (Wetmore 1968; Olson 1993), but it was subsequently split into a separate species (AOU 1995). Since its designation as a full species, the populations of the two putative species have not been found in either parapatry or sympatry, yet Olson (1993), in a survey of the birds of Bocas del Toro, Panama, identified allopatric populations in relatively close proximity (~40 km apart). Recent collections have not only identified a contact zone between the two putative species on the Pacific Slope of western Panama (3 km West of Remedios), but the two individuals of *A. prevostii* taken there represent the first records of the species on the Pacific Slope.

The syntopic occurrence of the two taxa suggests that they may indeed be full biological species, but this bears further investigation. In all phylogenetic reconstructions, *A. prevostii* was found to be part of a monophyletic clade that included *A. veraguensis*. Even though the two species did not share a common haplotype, only low levels of sequence divergence separated the two taxa. In addition to genotypic similarities, Schmitz-Ornés (2006) found moderate levels of overlap in plumage UV reflectance, causing predicted group membership to be misclassified in a discriminant analysis for 15% of males and 33% of females. Based on the monophyletic sister relationship found in our study and their phenotypic similarity, *A. veraguensis* may best be treated again as a subspecies of *A. prevostii* until further study where the two occur in sympatry can elucidate species limits.

GENES, MOVEMENT, AND BIOGEOGRAPHY

Phylogenies reconstructed from our full ND2 dataset recovered low levels of intraspecific sequence divergence both between geographically distant localities and within recognized subspecies. The largest within-species genetic divergences (1.15% in ND2) were recovered from the West Indian island endemic *A. dominicus*. In the case of *A. dominicus*, individuals from a single locality on the island of Hispaniola differed by three base pairs, whereas the largest phylogenetic split for the mainland taxa was only two base pairs, occurring between geographically distant specimens (~2800 km) separated by the Andes. Both the relatively high level of population genetic diversity in ND2 and the well-supported placement of *A. dominicus* as sister to all of the mangos (Figures 3.2, 3.3) suggest that the origin of the genus *Anthracothorax* occurred in the West Indies (Figure 3.4). The low levels of sequence divergence and placement of all mainland taxa as nested within a larger West Indian clade is suggestive of colonization of the mainland by island taxa (Figures 3.2-3.4).

The low levels of intraspecific divergence that we found can also be explained by the exceptional dispersal ability exhibited by members of the genus. This dispersal ability is most strikingly seen within *A. prevostii*. In 1988 the first United States record of this species was recorded in Corpus Christi, Texas, with subsequent verified records recorded in North Carolina in 2000 and Wisconsin in 2007 (LeGrand and Campbell 2005; Mastroianni 2007). In each of these cases, individuals were found ~750 to ~2600 km outside of the species' normal range. This dispersal ability is also seen within the West Indies. *Eulampis jugularis* occurs almost yearly on island of Grenada, which is nearly

150 km outside of the species' normal range. Each of these vagrant records suggests that geographic barriers such as intact forest, rivers, and mountains may not inhibit long-distance dispersal and gene flow.

Ancestral area reconstructions support a West Indian origin for all mainland members of *Anthracothorax* (Figure 3.4). As for the origin of the genus, parsimony and likelihood reconstructions also support a West Indian origin, but reconstructions using the Bayesian tree sampling methodology point to inconsistencies in the analyses. A virtually equivocal ancestral area was reconstructed for the genus over the 1000 post-burnin trees, but a West Indian ancestor was reconstructed for both the clade containing the entire genus except *A. dominicus* and the clade containing all of the mainland species. Alternate topologies supporting a one-way colonization of the West Indies were not supported by the data. Our data were thus equivocal regarding the origin of the genus *Anthracothorax*, but a West Indian ancestor was clearly supported for all mainland members of the genus. Thus, based on these data, West Indian members of the genus *Anthracothorax* successfully colonized the mainland. To accomplish this, *Anthracothorax* mangos had to overcome several obstacles that normally inhibit island-to-mainland colonization.

Several possible explanations for the rarity of island-to-mainland colonization have been proposed (Brown and Lomolino 1998; Cox and Moore 2005). One explanation relies on sheer numbers: colonizing populations from islands are limited by both the size of the source area and of the source population. With a smaller source population, fewer individuals arrive, and the probability of a population being large enough to resist stochastic extinction decreases. Another explanation is that because of their decreased

species richness, island fauna have not been subject to the more intense selective pressures present within more speciose mainland communities. Thus, selection on the mainland would favor the evolution of greater relative competitive abilities than selective pressures within less diverse island communities. As a result, the size of the island, the size of the source population, and the diversity of the source community may limit colonization of the mainland by island taxa while increasing the likelihood of mainland-to-island colonization.

Several aspects of their ecology and morphology have given *Anthracothorax* mangos a distinctive advantage in overcoming the obstacles associated with colonizing new areas. First is their proclivity of long-distance dispersal over land and water (Terborgh et al. 1978; LeGrand and Campbell 2005; Mastroianni 2007). The well-documented ability of *Anthracothorax* mangos to disperse outside of their normal ranges increases the probability of vagrants arriving in new habitats. Upon arrival, the ability to disperse over geographic barriers would enable individuals to move out of inhospitable environments that might inhibit establishment. Ecologically, the group's feeding habits also contribute to individual ecological flexibility. *Anthracothorax* mangos are known to hawk insects and rob nectar (Ornelas 1994; Schuchmann 1999). Because nectar robbing essentially allows the exploiter to bypass the coevolution often associated with flower morphology and hummingbird bill structure (Ornelas 1994), this adaptation could increase the likelihood of colonization of new areas by allowing food source flexibility. In addition, the aggressive nature and relatively large size of *Anthracothorax* species may help colonizers compete with previously established hummingbird fauna (Schuchmann

1999). Thus, the likelihood of successful colonization of the mainland by West Indian mangos may have been increased by the group's strong dispersal abilities, ecological flexibility, aggressive behavior, and large size.

CONCLUSIONS

Phylogenetic reconstructions indicate the need for several taxonomic revisions among *Anthracothorax* mangos and closely related genera. Both molecular and phenotypic evidence (other than plumage), support merging *Eulampis* into *Anthracothorax*, but the inclusion of the monotypic genus *Avocettula* is not supported. In addition, molecular data are not definitive regarding species status for the Panamanian endemic Veraguan mango; further study where they come into contact is warranted.

Biogeographically, ancestral area reconstructions could not confidently recover an origin for the genus as a whole, but they did support a West Indian origin for all mainland members of the genus. The radiations of *Anthracothorax* mangos out of the West Indies onto the mainland represent the first recognized example of mainland colonization by West Indian taxa for the family Trochilidae.

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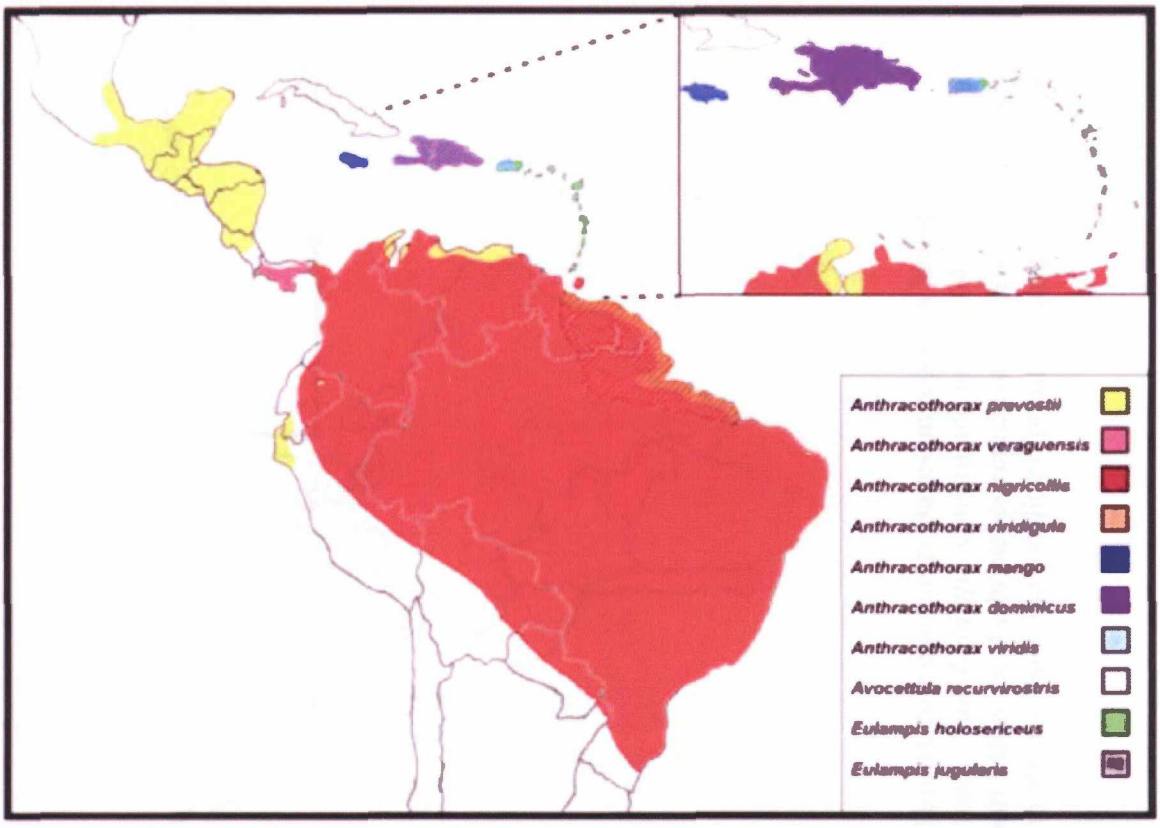

Figure 3.1 Geographic distributions of *Anthracothorax* and allied genera. Map of Middle and South America showing geographic distributions of species currently included in the genus *Anthracothorax* together with closely allied genera with contentious taxonomic relationships, *Avocettula* and *Eulampis*.

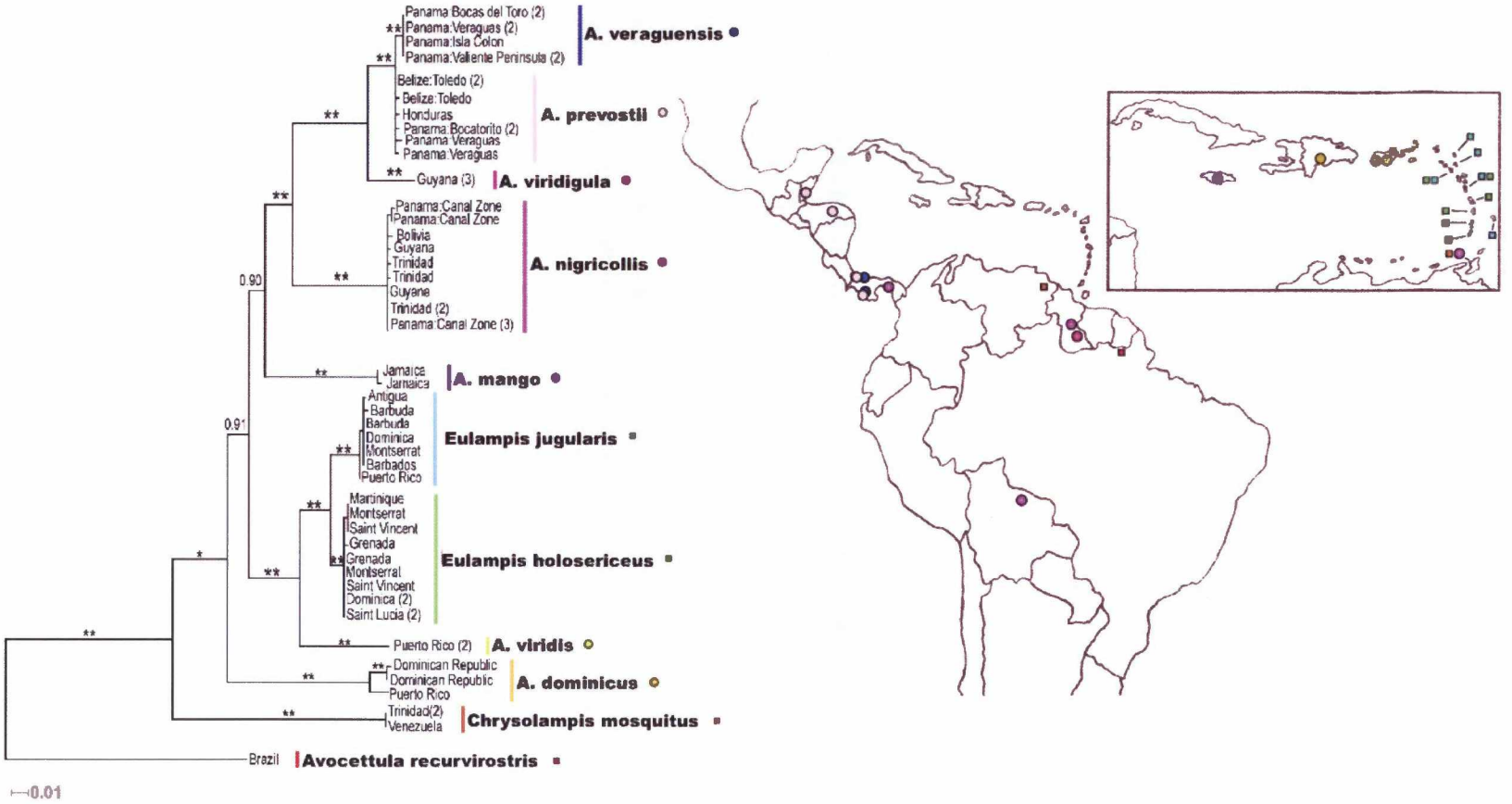

Figure 3.2 Bayesian ND2 phylogeny for *Anthracothorax*. Majority-rule consensus Bayesian topology based on 62 individuals of the genera *Anthracothorax*, *Avocettula*, *Chrysolampis*, and *Eulampis* constructed using 1041 bp of the mtDNA ND2 gene. Root (not shown) is to *Andron aequatorialis*. Support values on the branches represent posterior probabilities: double asterisk, 100%; single asterisk, 95% or greater. The map shows collection localities for the 62 samples, color coding

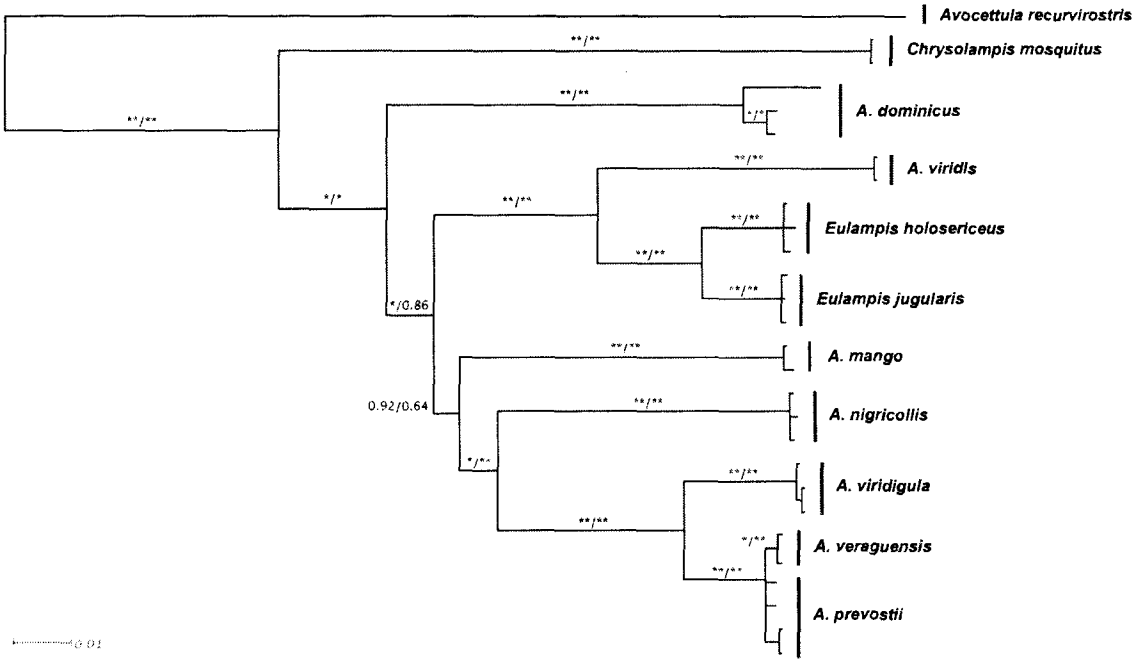

Figure 3.3 Full mitochondrial and nuclear DNA phylogeny for *Anthracothorax*. Majority-rule consensus Bayesian topology based on 28 individuals of the subsampled ND2 dataset, constructed using our full mitochondrial dataset (ND2, ATP-6 and -8, and COI-barcode: 2500 bp). Reconstruction of our five-sequence dataset (ND2, ATP-6 and -8, COI-barcode, Bfib5, and AK1) shared an identical topology, so support values from that analysis are included as the second support value on each branch. Support values are represented by posterior probabilities: double asterisk, 100%; single asterisk, 95% or greater. Root (not shown) is to *Andron aequatorialis*.

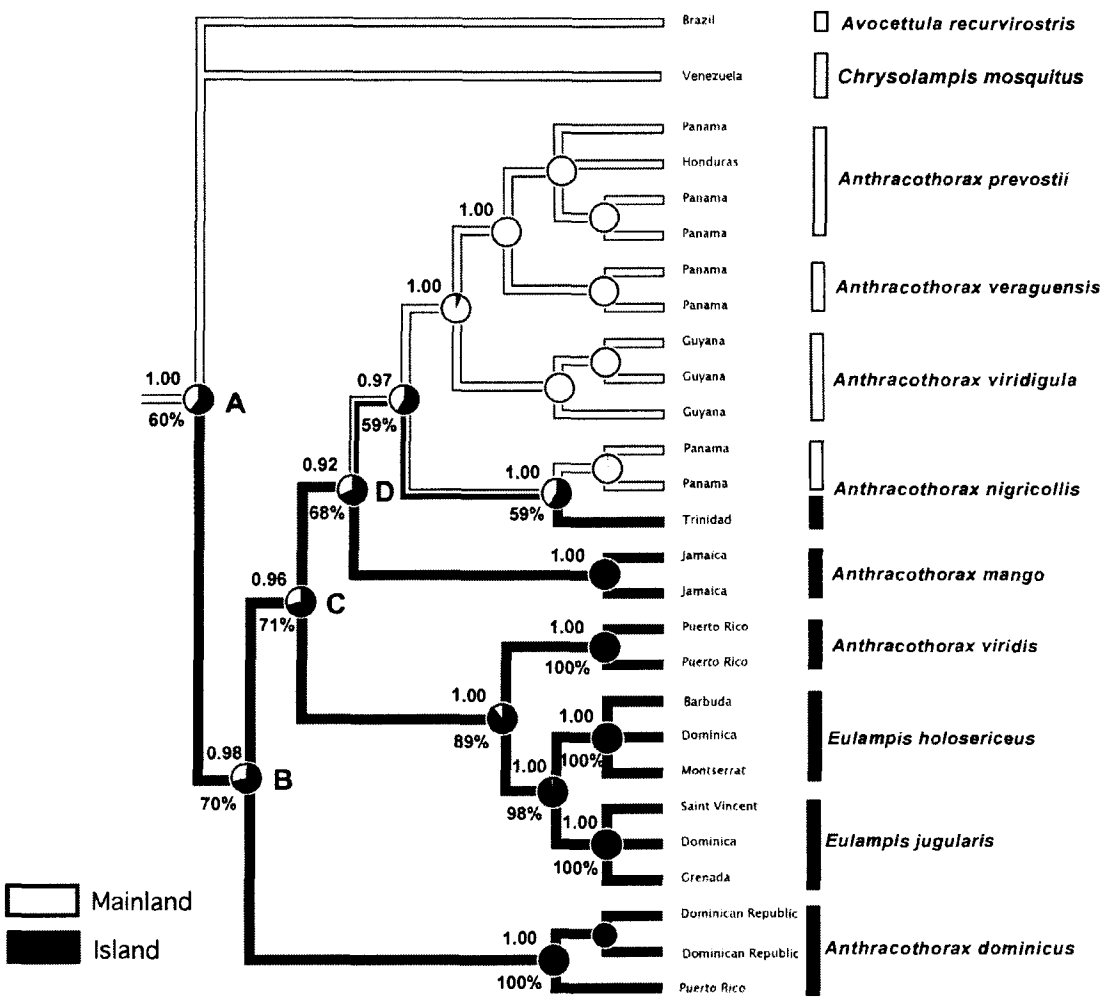

Figure 3.4 Ancestral area reconstruction. Ancestral area reconstruction for *Anthracothorax* mangos and closely allied genera. The phylogenetic tree represents the consensus Bayesian topology from our full mitochondrial dataset (ND2, ATP-6 and -8, and COI-barcode: 2500 bp). Posterior probabilities for each node are indicated above the branches. Branch shading indicates the most parsimonious state (Island or Mainland) for the branch, whereas circles at the nodes indicate relative likelihoods of each state. Relative likelihoods for all nodes with values greater than 50% reconstructing a mainland ancestor are placed under the branches. Basal nodes examined using Bayesian tree-search methodology are given a unique identifier (A, B, C, or D).

Appendix 3.1. Taxon, museum of origin, specimen catalog number, and geographic origin for birds in this study. Museum abbreviations are as follows: FMNH, Field Museum of Natural History; LSUMZ, Louisiana State University Museum of Natural Science; UNLV, University of Nevada, Las Vegas, Marjorie Barrick Museum of Natural History; UAM, University of Alaska Museum.

Taxon	Museum	Museum Number	Geographic Origin
Andron aequatorialis	STRI	EC-AAE2185	Ecuador:Alto Tambo
Avocettula recurvirostris	FMNH	334368	Brazil
Chrysolampis mosquitos	STRI	TR_CM01	Trinidad: Simia
C. mosquitos	STRI	TR_CM02	Trinidad: Simia
C. mosquitos	LSUMZ	B35902	Venezuela
Anthracothorax veraguensis	USNM	B1348	Panama:Bocas del Toro
A. veraguensis	USNM	B1349	Panama:Bocas del Toro
A. veraguensis	USNM	B1350	Panama:Valiente Penninsula
A. veraguensis	USNM	B1403	Panama:Bocas del Toro
A. veraguensis	USNM	B1777	Panama:Isla Colon
A. veraguensis	UAM	UAM24413	Panama:Chiriqui
A. veraguensis	UAM	UAM24414	Panama:Chiriqui
A. prevostii gracilirostris	UAM	UAM24638	Panama:Chiriqui
A. p. gracilirostris	UAM	UAM24639	Panama:Chiriqui
A. p. prevostii	UAM	UAM18200	Belize:Toledo
A. p. prevostii	UAM	UAM18202	Belize:Toledo
A. p. prevostii	UAM	UAM18201	Belize:Toledo
A. p. gracilirostris	USNM	B00360	Panama:Isla San Cristobal
A. p. gracilirostris	USNM	B00399	Panama:Isla San Cristobal
A. p. gracilirostris	STRI	HA_APR_HA270	Honduras:Cayo Cochino Pequeno
A. viridigula	FMNH	391259	Guyana:Karaudanawa
A. viridigula	FMNH	391260	Guyana:Karaudanawa
A. viridigula	FMNH	391261	Guyana:Karaudanawa
A. nigricollis	LSUMZ	B9552	Bolivia:Pando
A. nigricollis	MBM	GMS1883	Panama:Panama
A. nigricollis	MBM	GMS1886	Panama:Panama
A. nigricollis	MBM	GMS1888	Panama:Panama
A. nigricollis	MBM	GMS1890	Panama:Panama
A. nigricollis	MBM	GMS1972	Panama:Panama
A. nigricollis	FMNH	B12447	Guyana:Karaudanawa
A. nigricollis	FMNH	B12322	Guyana:Karaudanawa
A. nigricollis	STRI	TR_ANI1	Trinidad: Simia
A. nigricollis	STRI	TR_ANI4	Trinidad: Simia
A. nigricollis	STRI	TR_ANI10	Trinidad: Simia
A. nigricollis	STRI	TR_ANI11	Trinidad: Simia
A. mango	STRI	JA_AMA1	Jamaica
A. mango	STRI	JA_AMA2	Jamaica
A. dominicus dominicus	STRI	RD_AD01	Dominican Republic
A. d. dominicus	STRI	RD_AD02	Dominican Republic
A. d. aurulentus	STRI	PR_AD01	Puerto Rico
A. viridis	STRI	PR_AVR1	Puerto Rico
A. viridis	STRI	PR_AVR3	Puerto Rico
Eulampis holosericeus holosericeus	STRI	AN_SHO1	Antigua
E. h. holosericeus	STRI	BA_SHO2	Barbados
E. h. holosericeus	STRI	BU_SHO1	Barbuda
E. h. holosericeus	STRI	BU_SHO2	Barbuda
E. h. holosericeus	STRI	DO_SHO1	Dominica
E. h. holosericeus	STRI	MO_SHO1	Montserrat
E. h. holosericeus	STRI	PR_SHO1	Puerto Rico
E. jugularis	STRI	DO_EJU3	Dominica
E. jugularis	STRI	DO_EJU13	Dominica
E. jugularis	STRI	GU_EJU1	Guadeloupe
E. jugularis	STRI	GU_EJU2	Guadeloupe
E. jugularis	STRI	MA_EJU12	Martinique
E. jugularis	STRI	MO_EJU1	Montserrat
E. jugularis	STRI	MO_EJU13	Montserrat
E. jugularis	STRI	SL_EJU1	St. Lucia
E. jugularis	STRI	SL_EJU3	St. Lucia
E. jugularis	STRI	SV_EJU1	St. Vincent
E. jugularis	STRI	SV_EJU4	St. Vincent

GENERAL CONCLUSIONS

I used molecular genetic markers to examine historic patterns of differentiation associated with three groups of lowland Neotropical birds. In the first study, I found that *Amazilia tzacatl* is a monophyletic clade in relation to its closest relatives and exhibits four genetic clades: Atlantic and Pacific slopes of Middle America, South America, and Isla Coiba (the latter perhaps an early colonization of the Pacific Slope from the Atlantic Slope). To some extent the mtDNA clades corresponded with recognized subspecies, but there was disagreement as well, especially in the well-supported Atlantic and Pacific slope mtDNA clades, which are presently considered to be the nominate form *A. t. tzacatl*. The Escudo Hummingbird (*A. t. handleyi*), an endemic to Isla Escudo de Veraguas, Panama, and sometimes considered a separate species due to its larger size, did not have unique mtDNA haplotypes. Thus, the Escudo Island form is probably not a full biological species. Two contact zones were identified, both in Panama. One is between Atlantic Slope and Pacific Slope clades in Middle America, and the other, in the Darien, between Pacific Slope and South American clades; the latter appears to be recent. The syntopic occurrence in Darien, Panama of individuals from the deepest genetic split in the species suggests reproductive compatibility is retained between them. Thus, no cryptic species seem to occur within this taxon, neither phylogenetic nor biological.

In the second chapter I recovered an unresolved polytomy between *Henicorhina leucosticta* and its purported sister species, *H. leucoptera*. In the case of *Henicorhina leucosticta-leucoptera* wood-wrens, both the nested position of Middle American birds and the estimated divergence dates suggest a South American origin for this group.

Mitochondrial DNA haplotypes within *H. leucosticta* as presently recognized grouped into three clades: 1) Choco clade including individuals from northwestern Ecuador; 2) a Middle American clade from southern Mexico to Central Panama; and 3) An Amazon-Darien clade comprised of populations from northeastern Peru, eastern Ecuador, Guyana, and eastern Panama. In each of the three clades, both demographic analyses and molecular dating indicate that the final uplift of the Andes along with the closure of the Isthmus of Panama contributed to the relatively deep levels of sequence divergence between clades. In contrast to previous studies, we recovered high levels of structure among Middle American populations contradicting the hypothesis of a recent Middle American expansion. Taxonomically, it is possible that more than one biological species is involved in the *H. leucosticta* group. However, given the polytomous relationship between *leucosticta* and *leucoptera* and the inability to diagnose species limits based on mtDNA alone, we suggest further work be done to determine species limits among the four main clades of *leucoptera-leucosticta* complex.

In the third chapter, phylogenetic reconstructions indicate the need for several taxonomic revisions among *Anthracothorax* mangos and closely related genera. Both molecular and phenotypic evidence, support merging the genus *Eulampis* into *Anthracothorax*, but the inclusion of the monotypic genus *Avocettula* is not supported. In the case of the Panamanian endemic Veraguan mango, molecular data are not definitive regarding species status. Biogeographically, ancestral area reconstructions could not confidently recover an origin for the genus, but they did support a West Indian origin for all mainland members of the genus. The radiations of *Anthracothorax* mangos out of the

West Indies on to the mainland represent the first recognized example of mainland colonization by West Indian taxa for the family Trochilidae.